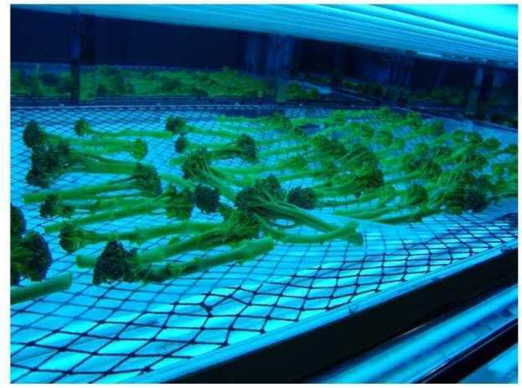

Effects of alternative sanitizing techniques on nutritional quality of fresh-cut Bimi® broccoli during shelf life

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Todas as correcções determinadas pelo júri, e só essas, foram efectuadas.

O Presidente do Júri,

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Porto, 10/01/2013

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EFFECTS OF ALTERNATIVE SANITIZING TECHNIQUES ON NUTRITIONAL QUALITY OF FRESH-CUT BIMI® BROCCOLI DURING SHELF LIFE

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Dissertation presented by Anna Carolina Formica de Oliveira, in partial fulfillment of the requirements for the Master of Sciences in Agronomic Engineering at the Faculty of Sciences of the University of Porto

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ABSTRACT

In the present days, consumers are faced with a diverse variety of minimally processed fruits and vegetables, where sensorial and nutritional quality plays a major role on marketing those products. Vegetables of the *Brassicaceae* family are rich in polyphenols, flavonoids and glucosinolates, among other nutrients; however broccoli has high respiration and transpiration rates, and a short shelf life. Bimi® broccoli (*B. oleracea* Italica x Alboglabra) is a green vegetable similar to broccoli but with small florets and long stalks. This new variety possesses a characteristic flavor and makes it much appreciated by the consumer. Although chlorine is the most widely sanitizer used in fresh-cut vegetables, it has some disadvantages that lead to research on alternative treatments. The treatments were applied single (superatmospheric, designed also high, oxygen active modified atmosphere packaging (MAP) – HO_2; Neutral Electrolyzed Water- NEW; Ultraviolet C radiation- UV-C) and combined (a mix of electrolyzed water and radiation- NEW+UV-C; a combination of neutral electrolyzed water and high oxygen MAP - NEW+HO_2; UV-C radiation plus high oxygen MAP- UV-C+HO_2; and the triple combination - NEW+UV-C+HO_2 [MIX]). As control, 100 ppm NaClO was used as sanitizer in the washing step and another washed with tap water was used as well. After fresh-cut processing, Bimi® was stored at 5°C in darkness and total antioxidant capacity (FRAP and DPPH assays), total polyphenol content and fatty acid quantification were analyzed on the processing day and after 5, 9, 15 and 19 days at 5°C. On the subject of polyphenols content, sanitizing treatments reduced the initial total polyphenols amount of 1,392 mg ChAE kg^{-1} fw in approximately 13-14% on the processing day, without differences among treatments. Generally throughout storage, an initial total polyphenols capacity decrease was observed until day 15. From day 15th until the end of storage period an increase was registered. Regarding antioxidant capacity (FRAP assay), all treatments decreased the total amount of antioxidant capacity after 5 days, but subsequently, an increased was found after 9 days at 5ºC. On the processing day, Control showed 722.3 mg AAE kg^{-1} fw, nonetheless presented the higher decrease after 15 days (257.1 mg AAE kg^{-1} fw). On the other hand, for DPPH method, the initial total antioxidant capacity content in all treatments was quite similar, however a high decrease was found after 19 days of storage. For fatty acid content, three main fatty acids in Bimi® were quantified (linolenic, stearic and palmitic acids). Control samples showed 8.43µmol g^{-1} fw of linolenic acid on the processing day, but showed the highest decrease on its content during shelf-life, mainly caused by lipid peroxidation. With UV-C, the initial content was 5.50 µmol g^{-1} fw. Nevertheless, presented the lowest decrease over storage, as well as with

superatmospheric O_2, although without significant differences ($p>0.05$). Regarding stearic acid content, all treatments responded with stable or slightly increasing values. While for palmitic acid content, NEW treatment responded with initial content of 2.23 µmol g^{-1} fw, conversely, presented a decrease of 10% on its content throughout storage, with all other treatments, remaining constant. As main conclusion, for polyphenol content, all treatments had similar activities, although UV-C treatment demonstrated stable or lightly increasing values after 19 days of shelf life, and could be remarked the best treatment for keeping the total polyphenols volume at 5ºC regarding the initial control values. For the FRAP assay could be concluded that all treatments had similar activities, although the NEW treatment demonstrated stable or lightly increasing values after 9 days of shelf life, and could be remarked the best treatment for keeping the antioxidant capacity at 5ºC. On the other hand, about DPPH assay as main conclusion, even though all treatments had a similar trend, the combination of NEW+UV-C+HO_2 could be remarked the best treatment for keeping the total antioxidant capacity at 5°C. As a main conclusion, linolenic acid was the main fatty acid quantified in Bimi®, with a stable or slightly increasing trend during refrigerated shelf-life, after different sanitizing treatments. Superatmospheric O_2 presented a higher amount throughout shelf-life (2.68 µmol g^{-1} fw), being marked as the best treatment to preserve the set of fatty acids under evaluation.

RESUMO

Os hábitos alimentares estão a ser modificados ao longo das últimas décadas. O actual ritmo de vida, com escasso tempo para preparar refeições equilibradas provocou a demanda de produtos vegetais naturais, frescos e saudáveis, prontos ao consumo, como os minimamente processados em fresco (MPF), denominados comercialmente “IV gama” da alimentação. Os vegetais da família *Brassicaceae* são ricos em polifenóis, flavonóides e glucosinolatos, assim como outras substâncias benéficas ao homem. No entanto os brócolos possuem altas taxas de respiração e transpiração, factores que encurtam a sua vida útil de produto alimentar. Bimi® (*B. oleracea* Italica x Alboglabra) é um vegetal similar aos brócolos convencionais, com floretes mais pequenos e talo alongado. Apresenta-se como uma alternativa, pois possui um sabor característico muito apreciado pelo consumidor. O hipoclorito de sódio (NaClO) actualmente é o desinfectante mais utilizado nos produtos minimamente processados, porém possui algumas desvantagens que conduziram a procura de novas alternativas mais seguras e amigas do ambiente. Os tratamentos desinfectantes foram aplicados sozinhos (Oxigénio superatmosférico em embalagem modificada activa (MAP) – HO_2; Água electrolisada neutra – AEN; Radiação ultravioleta C – UV-C) e combinados (misturas de água electrolisada neutra e radiação – AEN+UV-C; combinação de água electrolisada neutra e oxigénio superatmosférico MAP – AEN+HO_2; radiação UV-C mais oxigénio superatmosférico UV-C+HO_2 e finalmente a combinação tripla AEN+UV-C+HO_2 [MIX]). Como controlo, dois tratamentos foram utilizados: um com 100ppm de NaClO no processo de lavagem, e outro contendo água potável. Após o processamento mínimo, Bimi® foi armazenado a 5°C em total ausência de luz e ensaios de capacidade antioxidante (métodos DPPH e FRAP), conteúdo total de polifenóis e quantificação de ácidos gordos, foram analisados no dia de processamento e após 5, 9, 15 e 19 dias a 5°C. Relativamente ao conteúdo de polifenóis, os tratamentos desinfectantes reduziram o conteúdo total de 1,392 mg EAC kg^{-1} pf em aproximadamente 13-14% no dia do processamento, sem diferenças entre tratamentos. Durante o período de armazenamento, foi-se observado um decréscimo no conteúdo de polifenóis até o 15º dia. Reversamente, a partir deste dia até o fim da vida útil um aumento foi registado. No ensaio de capacidade antioxidante (método FRAP), todos os tratamentos reduziram a quantidade total após 5 dias, porém subsequentemente, um aumento foi observado após 9 dias de armazenamento. No dia do processamento, o controlo possuiu 722.3 mg EAA kg^{-1} pf, no entanto apresentou a maior queda após 15 dias (257.1 mg EAA kg^{-1} pf). Em contrapartida, para o ensaio com o método DPPH, a capacidade antioxidante inicial em todos os

tratamentos foi similar, contudo um grande decréscimo foi encontrado após 15 dias de vida útil do produto. Para o conteúdo de ácidos gordos, os três principais foram quantificados (ácidos linolénico, esteárico e palmítico). As amostras controlo mostraram 8.43µmol g^{-1} pf de ácido linolénico no dia de processamento, entretanto teve a maior descensão no seu conteúdo durante os 19 dias de armazenamento, causado principalmente pela peroxidação lipídica. Relativamente às amostras tratadas com radiação UV-C, o conteúdo inicial foi de 5.50 µmol g^{-1} pf, onde apresentaram a menor queda, assim como as tratadas com oxigénio superatmosférico, sem diferenças significativas ($p>0.05$) entre as mesmas. No que respeita ao conteúdo de ácido esteárico, todos os tratamentos responderam com valores estáveis ou ligeiramente elevados. Entretanto, para o conteúdo de ácido palmítico, AEN obteve um conteúdo inicial de 2.23 µmol g^{-1} pf, e contrariamente apresentou uma diminuição de aproximadamente 10% no seu conteúdo, onde todos os outros tratamentos permaneceram constantes. Como principal conclusão, para o conteúdo total de polifenóis, todos os tratamentos tiveram actividades similares, no entanto o tratamento UV-C demonstrou valores estáveis ou mesmo ligeiramente acrescidos após 19 dias de vida útil, e pode ser considerado o melhor tratamento para manter o volume total de polifenóis a 5°C, com respeito aos valores iniciais de controlo. Para o método FRAP, pode-se concluir que apesar de todos os tratamentos apresentarem um comportamento similar, AEN obteve valores estáveis e ligeiramente acumulados e é considerado o melhor tratamento para manter a capacidade antioxidante a 5°C. Por outro lado, no método DPPH, como conclusão destaca-se que mesmo tendo tendências similares, a combinação NEW+UV-C+HO_2 foi o tratamento que melhor manteve a actividade antioxidante a 5°C. Relativamente ao ácido linolénico, apresentou-se como o principal ácido gordo da couve-brócolo Bimi® com um comportamento estável durante o armazenamento refrigerado, após diferentes métodos desinfectantes. O tratamento com oxigénio superatmosférico exibiu o maior volume durante a vida útil (2.68 µmol g^{-1} pf), sendo assinalado como o melhor tratamento para preservar o conjunto de ácidos gordos estudados.

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AA: Ascorbic Acid

AAE: Ascorbic Acid Equivalent

AEN: *Água Electrolisada Neutra*

AEW: Acidic Electrolyzed Water

BHT: Butylated hydroxytoluene

BOPP: Micro perforated bi-oriented polypropylene film

CAS: Controlled atmosphere storage

ChAE: Chlorogenic Acid Equivalent

DPPH: 2,2-Diphenyl-1-picrylhydrazyl, free radical

EAA: *Equivalente de Ácido Ascórbico*

EAC: *Equivalente de Ácido Clorogénico*

ETC: Electron transport chain

EW: Electrolyzed Water

FDA: US Food and Drug Administration

FRAP: Ferric Reducing Ability of Plasma

GC: Gas chromatography

GPR: *Grupo de Postrecolección y Refrigeración*

HO_2: Superatmospheric (high) oxygen

HPLC: High-performance (pressure) liquid chromatography

ICP: Inductively Coupled Plasma

MAP: Modified Atmosphere Packing

MPF: Minimally processed foods (Fresh-cut)

MS: Mass spectrometry

MUFA: Monounsaturated Fatty Acids

NEW: Neutral Electrolyzed Water

PAL: Phenylalanine ammonia-lyase

PP: Polypropylene

PUFA: Polyunsaturated Fatty Acids

ROS: Reactive Oxygen Species

SD: Standard Deviation

SE: Standard Error

SFA: Saturated Fatty Acids

TPTZ: 2,4,6-tripyridy-s-triazine

UK: United Kingdom

UPCT: Technical University of Cartagena

USA: United States of America

UV-C: non-ionizing UV-C radiation

STATE OF THE ART

Today's society is characterized by an increasing health consciousness and growing interest in the role of food for maintaining and improving human well-being and consumer health. Vegetables and fruits are fully recognized for their benefits towards healthy living thanks to their protective function against cancer and other chronic degenerative diseases (Ragaert, Verbeke, Devlieghere, & Debevere, 2004)

The current lifestyle with reduced time to prepare health meals has prompted the high demand of fresh, natural and ready-to-eat fruits and vegetables. Convenience is a major concern in food purchases, particularly by members of urbanized societies. Consumers' convenience orientation not only relates to physical activities but also to thinking activities involved in meal preparation (De Moura & Cunha, 2005).

Cruciferous or *Brassica* vegetables are so named because they come from plants in the family known to botanists as *Brassicaceae* or alternately, *Crucifera*, in allusion of a cross design on four petals in their flowers. It's a well known family with about 350 genus and 3000 species, mostly on temperate zones, especially on Mediterranean Sea. Also, it has a wide use on culinary, including species such as broccoli, Brussels sprouts, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard, rutabaga, turnips, bok choy, and Chinese cabbage. These Brassica vegetables are rich in a number of biologically active compounds such as vitamins, phenolic acids, flavonoids and glucosinolates, which are associated with antioxidant, antibacterial and anticancer properties (Jaiswal, Rajauria, Abu-Ghannam, & Gupta, 2011)

Broccoli has high respiration and transpiration rates, and a short shelf life. It is very temperature-sensitive, so that storage at high temperatures leads to accelerated quality loss due to wilting and yellowing (Murcia, López-Ayerra, & García-Carmona, 1999).

Enhancing the health benefit properties of fruits and vegetables will add value and create new opportunities for growers and processors by reaching health-oriented markets. To achieve this goal, there is a need to provide technologies that can ensure the delivery of high quality products with high levels of the desired nutraceuticals (Cisneros-Zevallos, 2003).

1.1 Bimi® Broccoli

Bimi® broccoli, a new commercial broccoli variety (also called as tenderstem®, vellaverde®, broccolini®, asparation, inspiration, broccoletti or broccolette) is a hybrid between conventional broccoli (*Brassica oleracea*, Italica group) and Chinese broccoli (*B.*

oleracea, Alboglabra group, also called kai-lan or Chinese kale). New varieties of broccoli with less intense flavor than the conventional ones are appearing in the international market in order to increase their consumption. Bimi® looks like conventional broccoli with a long slender stem, but has a milder sweeter taste similar to green asparagus (Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011)

The origin of Bimi® dated back to 1994, when Sanbon Incorporated originated a commercial program for asparation in Mexico and first brought it to the US market in 1996. Two years later, Mann Packing Company partnered with Sakata Seeds Co. began to market the new vegetable throughout US under the name of broccolini® (Mount Vernon Northwestern Washington Research and Extension Center, 2010).

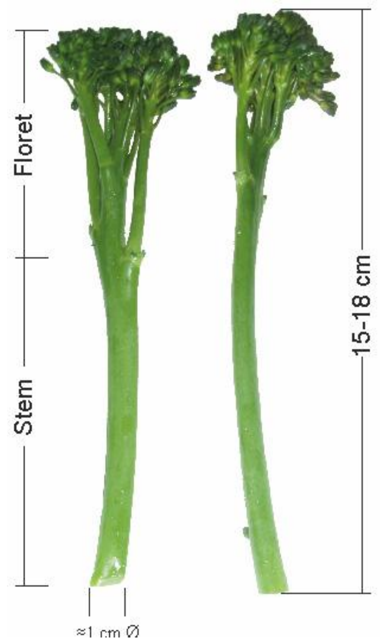

Fig. 1 - *Visual appearance of Bimi® broccoli* (Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011).

While broccoli is harvested three times during a season, Bimi® may be harvested 3–5 times depending on growing conditions. Both removing the main stalk and harvesting the side shoots is much more labor intensive than broccoli production. The hybrid is also intolerant of temperature extremes and more sensitive to cold temperatures than broccoli (Mount Vernon Northwestern Washington Research and Extension Center, 2010).

Bimi® contains ten essential nutrients and has more zinc, folic acid, anti oxidants, glucosinolates combined than asparagus, traditional broccoli, curly kale and spinach. Considered by some a super food, this vegetable contains as much vitamin C as orange juice and provides substantial amounts of vitamin A and potassium. It has some iron, calcium, vitamin B and fiber as well (Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011).

Table 1 – *Nutrition facts sheet of Bimi® broccoli.*

Nutrition Facts		
Serving size about 8 stalks (82 grams)		
	Amount Per Serving	**%Daily Value**
Calories	35	
Total fat	0g	0%
Sodium	25mg	1%
Total Carbohydrate	6g	2%
Cholesterol	0mg	0%
Potassium	270mg	8%
Protein	3g	
Vitamin A 30%		Vitamin C 130%
Calcium 6%		Iron 4%

(Adapted from MannPacking Co, 2012)

1.2 Minimally processed (Fresh-Cut) produce

Fresh-cut, initially called minimally processed or lightly processed products can be defined any fresh fruit or vegetable that has been physically modified from its original form (by peeling, trimming, washing and cutting) to obtain 100% edible product that can be subsequently bagged or prepackaged and kept in refrigerated storage. In those aspects, could include any kind of fresh commodities and their mixtures in different cuts and packaging. And according to the United Fresh Produce Association, minimally processed produce are defined as any fruit or vegetable that has been altered physically from its original, but retains its status fresh (Martín-Belloso & Soliva-Fortuny, 2010).

After storage for a certain period, one or more quality attributes of a food may reach an undesirable state. At that time, the food is considered unsuitable for consumption and is said to have reached the end of its shelf life. This level is defined by the manufacturer according to criteria when the product is saleable. Various approaches have been used to control the undesirable physiological changes that adversely affect the quality of minimally processed products. Refrigeration, humidity control, and dipping in chemical solutions such as ascorbic acid and calcium have been used successfully to preserve product quality and enhance shelf life (Shafiur Rahman, 2007).

Fresh-cut products have been available for many years, but the types and quantity have expanded tremendously in the past decade. Initially, the food service industry (e.g., institutions) was the main user of fresh-cut products, but use has expanded to restaurants, supermarkets, and warehouse stores (Watada, Ko, & Minott, 1996).

Fig. 2 – *Supermarket shelf of Fresh-Cut vegetables (Cadena Hortofruticola, 2009).*

In Europe, the minimally processed vegetables were first introduced by Florette Group in the early 80's for the French market, and subsequently exporting to United Kingdom, Italy and Switzerland. This is a growing industry in European Union, where United Kingdom, France and Italy share leaders. Nevertheless, the countries with higher growth in fresh-cut products market are Germany, Netherlands, Spain and United Kingdom. On the other hand, fresh-cut produce have been adapted to each country according to consumer preferences, production, distribution and legislation (Martín-Belloso & Soliva-Fortuny, 2010).

Knowledge of the nature of fresh-cut fruits and vegetables, as they relate to pre- and postharvest handling, processing, packaging, and storage, is essential for ensuring their safety, nutritional value, and maintaining quality. Fresh-cut produce are more perishable than intact vegetables because they have been subjected to several physical stress, such as cutting, slicing, peeling, shredding, trimming, and coring (Iqbal, et al., 2008). Minimally processed fresh (MPF) fruits and vegetables provide a good substrate for microbial development. Presence of cut surfaces and the high moisture content in packages increases possible spoilage by microorganisms. Contamination of MPF fruits and vegetables can occur at most steps of the food chain, from cultivation tom processing. Contamination risk increases with a polluted environment during cultivation or poor hygienic conditions in processing (Ayhan, Chism, & Richter, 1998)

1.3 Sanitizing treatments

In recent years, producers, regulatory agencies, and the public have become increasingly concerned about the microbiological safety of fruits and vegetables. Produce-related outbreaks of foodborne illness are more numerous. Outbreaks have been attributed to sprouted seeds, leafy vegetables, tomatoes, melons, berries, and unpasteurized juices (Sapers, 2001). In order to achieve fresh-cut produce with fresh-like quality, safety and high nutritional value, the industry needs to implement improved strategies by introducing or combining sustainable techniques, especially standard procedures for sanitation (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009).

It is well known that fresh-cut processors usually rely on wash-water sanitizers to reduce initial bacterial populations in their fresh-cut products as a strategy to maintain their quality and extend their shelf life (Gil, Selma, López-Gálvez, & Allende, 2009). A variety of methods are used to reduce the potential for microbial contamination. Each method has distinct advantages and limitations depending upon the type of produce, mitigation protocol, and other variables (Martín-Belloso & Soliva-Fortuny, 2010). Methods of cleaning and sanitizing produce surfaces normally entail the application of water, cleaning chemicals (for example, detergent), and mechanical treatment of the surface by brush or spray washers, followed by rinsing with potable water (Artés & Allende, 2005).

Chlorine-based chemicals, particularly liquid chlorine and hypochlorite, are probably the most widely used sanitizers for decontaminating fresh produce (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). To disinfect produce, sodium hypochlorite is commonly used at concentrations of 50-200 ppm with a contact time of 1-2 minutes (Beuchat, 1998). The antimicrobial activity of chlorine compounds depends on the quantity of hypochlorous acid (HOCl) current in the water which depends on the pH of the water, the concentration of organic material in the water, and, to some extent, the temperature of the water (Martín-Belloso & Soliva-Fortuny, 2010).

Nevertheless, in recent times, the outbreaks associated with food-borne pathogen contamination in fresh-cut vegetables elevated the concerns about the efficacy of chlorine as sanitizer in assuring the safety of the products (Ölmez & Kretzschmar, 2009). Sodium hypochlorite (NaOCl) can incompletely oxidize food components that contain natural organic materials to create harmful by-products in process water, such as chloroform ($CHCl_3$) or haloacetic acids that have known or suspected carcinogenic or mutagenic properties, with proven toxicity to human organs (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009). For that reason, became imperative to study more environmental friendly, as well as healthy safe alternative techniques from chlorine.

Recently, disinfection with electrolyzed water has been considered as an eco-innovative sanitizer technique that could be used to replace chlorine in the fresh-cut industry. There are two types of electrolyzed water with sanitizing properties: acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW). These solutions are generated by electrolysis of a diluted (0.5–1.0%) salt (NaCl) solution. The solution is split into two channels, one running through the anode (+) chamber and the other through the cathode (−) chamber where it is exposed to a controlled electrical potential difference. It causes an enrichment in the anode chamber of chlorine ions, forming an anolyte solution (AEW) and an enrichment in the cathode chamber of sodium and hydroxide ions, forming a catholyte solution (NEW) (Graça, Abadias, Salazar, & Nunes, 2011). Since the mid 1980s, EW has been certified for use in Japan as a medical product. The first form of EW that was developed was the acidic type, and it was accepted quickly by the food industry in Japan. It was found to be useful in killing bacteria and parasites on raw fish without altering sensory characteristics (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009).

Because of its neutral pH, NEW does not contribute as aggressively as AEW to the corrosion of processing equipment or irritation of hands, and is also more stable as chlorine loss is significantly reduced at pH 6–9 (Deza, Araujo, & Garrido, 2003). In addition, when EW comes in contact with organic matter, or is diluted by tap water or reverse osmosis water, it becomes ordinary water again. Thus, it is more eco-friendly than NaClO and is not potentially harmful for human health. However, there is little information available on the effects of NEW and AEW on the main quality attributes in fresh-cut vegetables (Tomás-Callejas, Martínez-Hernández, Artés, & Artés-Hernández, 2011).

Ultraviolet radiation (UV) is a type of non-ionzing radiation with wavelengths from 100 to 400nm, which is usually classified as UV-A (315-400nm), UV-B (280-315) and UV-C (100-280nm). UV-C irradiation is, of the three above cited, the one with highest germicidal action and therefore more studied (Gutiérrez-López, Barbosa-Cánovas, Welti-Chanes, & Parada-Arias, 2008)

Among the possibilities, disinfection by UV-C radiation is interesting, because no residues are released and the cost is relatively low (Artés-Hernández, Escalona, Robles, Martínez-Hernández, & Artés, 2009). UV-C light in the range of 240–260 nm has been approved in the USA to be used in food as a surface sanitizing treatment (FDA, US Food and Drug Administration, 2001), being as effective as NaClO or O_3 (Artés-Hernández, Robles, Gómez, Tomás-Callejas, & Artés, 2010). Also, exposure to low UV-C radiation doses has been reported to reduce postharvest decay of many horticultural crops (Allende & Artés, 2003). Some authors suggested for as the growing demand for ‘organic’ foods, the potential use of UV-C as an alternative to fungicides for the control of post-harvest diseases of stored vegetables such as carrots has attracted attention (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000).

UV light induces both biological stress and defense mechanisms of plant tissues. These inducible effects include the accumulation of antimicrobial compounds cell wall modification, an increase in the activity of defense enzymes, and increased antioxidant activity. All of these changes empower the plant tissue to resist decay (Alothman, Bhat, & Karim, 2009). Also, Artés, Gómez, Aguayo, Escalona, & Artés-Hernández (2009), verified that abiotic stresses such as that from UV-C light may enhance the nutraceutical content of fresh fruit and vegetables. It would affect secondary metabolism of fresh produce and would increase synthesis of phytochemicals with nutraceutical activity or reduce synthesis of undesirable compounds. The crucial point is whether a safe dose could be found that would greatly impair pathogen growth without damaging the product (Artés-Hernández, Escalona, Robles, Martínez-Hernández, & Artés, 2009).

Allende & Artés (2003), however, cited that UV radiation has been recommended as best used in combination with other preservation techniques, since the accumulative damage due to microbial DNA appears effective decreasing the overall number of bacteria cells, but does not result in complete sterilization. For that reason, in the present research combined treatments using UV-C radiation were applied to verify its influence over nutritional quality through shelf life.

A modified atmosphere can be defined as one that is created by altering the normal composition of air (78% nitrogen, 21% oxygen, 0.03% carbon dioxide and traces of noble gases) to provide an optimum atmosphere for increasing the storage length and quality of food/produce (Hui, 2003). This can be achieved by using controlled atmosphere storage (CAS) and/or active or passive modified atmosphere packaging (MAP) (FDA, US Food and Drug Administration, 2001). Low levels are typically applied to reduce respiration and the associated quality loss of vegetable and fruit produce (Geysen, Verlinden, Geeraerd, Van Impe, Michiels, & Nicolaï, 2005).

A novel type of MAP using superatmospheric oxygen has been described as effective to inhibit enzymatic browning, prevent anaerobic fermentation and moisture and odor losses and reduce aerobic and anaerobic microbial growth (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009). However some authors verified atmospheres with elevated oxygen may influence the synthesis and accumulation of some volatiles compounds associated with respiratory metabolism. Still there are few or very little information available on effects of superatmospheric oxygen in concentration of nutritional compounds in fresh-cut produce (Kader & Ben-Yehoshua, 2000).

Hurdle technology is the combination of different preservation techniques as a preservation strategy. The most important hurdles commonly used in food preservation are based on controlling temperature, water activity, acidity, redox potential and the use of preservatives, modified atmosphere and competitive microorganisms (e.g., lactic acid bacteria) (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). It would be expected that combinations of sanitizers and other intervention methods, such as heat or irradiation, would have additive, synergistic, or antagonistic interactions (Allende, Tomás-Barberán, & Gil, 2006). Nevertheless, more studies should be carried to verify the influence of combined techniques on food safety of fresh-cut fruits and vegetables.

1.4 Importance of healthy nutrition and its influence over fresh-cut produce

Nowadays, assets have been distributed by both private and public sectors to educate the population over the nutrition information programs. Some of the commonly known public nutrition information or promotion programs include the Recommended Daily

Allowances, Food Pyramid, the Surgeon General's Report on Diet and Health, the Five-a-Day for Better Health, and the Dietary Guidelines for Americans (Nayga, 1997).

Good nutrition however is strongly linked to good health. For that reason, firstly, the concept of health and good health should be demystified. According to De Moura & Cunha, (2005), health is not simply the absence of illness or injury, and is an important part of well-being, of how people feel and function, and also contributes to social and economic well-being.

Conventionally, recommended healthy alimentation was focused on the intake of food that increase energy levels and/or avoid mineral deficiencies. Moreover, nowadays the trend was updated for the concept of dietary recommendations and guidelines that may reflect growing concern about diet-related non-communicable diseases, and recommendations now frequently include alerts and notes regarding intake of those dietary components that are associated with increased/decreased risk of these diseases (De Moura & Cunha, 2005).

There is a general agreement that the high intake of fruits and vegetables can help prevent wide range of chronic diet related diseases including cardiovascular diseases and cancers (Cox, Reynolds, Mela, Anderson, McKellar, & Lean, 1996). Additionally, various public organisms recommend daily doses, such as 400g of fruits and vegetables (WHO Technical Report Series 916, 2003) and in developed countries campaigns to incentive a healthy lifestyle have been launched (Ragaert, Verbeke, Devlieghere, & Debevere, 2004).

Another major point that researches should have in mind, when studying nutrition, is the changes in lifestyle over the population. According to another study led by Cunha & De Moura (2004), modern consumers are increasingly demanding convenience, as there is less time available to cook from raw materials, and also variety of products. An additional concern is food safety, where foodstuff should be free of pathogens, chemicals residues and have not been produced from harmful genetic modification.

The nutritional quality and health-beneficial properties of plant products have been studied by many research groups because of the growing consumer interest in recent years in including functional foods in their diets (Martín-Belloso & Soliva-Fortuny, 2010). Functional foods could be described any food that contain highest levels of nutrients, in other words, food that have healthy benefits beyond normal nutrition (Abdel-Salam, 2010).

A global processing and storage design to achieve high-quality minimally processed foods requires a combination of different strategies and technologies to help reduce degradative processes in fresh-cut vegetables. Moreover, the content of bioactive compounds in minimally processed plant products can vary with genotype, environmental stress, growth conditions, and storage and processing conditions (Martín-Belloso & Soliva-Fortuny, 2010).

Cisneros-Zevallos (2003) cited that there are many studies which indicate that there is a potential in using postharvest stresses to induce accumulation of targeted phytochemicals, thereby enhancing the genetic potential of fruits and vegetables and yielding products with increased health benefit properties. However, there are only few references in the literature reporting the use of controlled stresses to enhance the accumulation of nutraceuticals, well known compounds with high nutritional qualities.

As cited before, processing may affect, to a significant extent, the concentration and biological activities of different compounds present in plants. This aspect seems to be very important taking into account that only some vegetables are consumed in a raw state and most of them are processed before consumption (Gliszczyńska-Świgło, Ciska, Pawlak-Lemańska, Chmielewski, Borkowski, & Tyrakowska, 2006). Among the considerations described above, this study specifically concentrates the research on total polyphenol content, antioxidant capacity and fatty acids levels that has proven importance over a healthy nutrition.

1.4.1 Fatty Acids

Fatty acids are simple in structure, with a carboxyl group at one end and a methyl group at the other end of a carbon backbone. This backbone usually ranges between 6 and 24 carbons in length and is generally even-numbered. Their nomenclature is derived from the number of carbon atoms, the number of double bonds and the position of the first double bond on the carbon chain opposite the carboxyl group. Many but not all fatty acids can be synthesized, lengthened or desaturated endogenously (Arab, 2003).

Those compounds are the basic structural components of triglycerides and are also found in phospholipids and cholesterol esters. They are rarely available as free fatty acids in vivo. Fats have important functions as storage units for energy, as structural units in membranes and as precursors to eicosanoids. They can therefore be found in serum, membranes and adipocytes. The proportion to be found in each media and molecule appears to depend upon the type of fatty acid (Arab, 2003). For example, long-chain Polyunsaturated Fatty Acids (PUFA), especially those of the ϖ-3 series and α-linolenic (18:3 n-3), are essential for human metabolism and have many beneficial effects including the prevention of a number of diseases, such as coronary heart diseases, inflammation, autoimmune disorders, hypertension, hypotriglyceridemic effect, cancer, etc (Arnaíz, Bernal, Martín, García-Viguera, Bernal, & Toribio, 2011).

Fatty acids which have all the carbon atoms in the chain linked by single bonds are called Saturated Fatty Acids (SFA). Most dietary SFA are 12–18 carbon atoms long, solid at room temperature (21°C) and relatively insoluble in water. Palmitic acid (C16:0) and stearic acid (C18:0) are the most commonly occurring dietary SFA accounting for approximately 50% and 24%, respectively of UK SFA intakes. Mammals have the ability to endogenously synthesize SFA and Monounsaturated Fatty Acids (MUFA), and therefore their inclusion into the diet is not considered 'essential'. However, the PUFAs, linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are essential nutrients because they cannot be synthesized in mammalian tissues and are required for normal physiological functioning (Mason, et al., 2009), as before mentioned.

Although dietary fat is an essential component of our diet, it is often labeled as the 'villain' of nutrients because of the link between dietary fat and the risk of a number of chronic diseases. However, not all dietary fat is equal with regard to its health effects and it is now recognized that modification of the type of fat, rather than a reduction in the total amount of fat in the diet, may have a greater impact on manipulating disease risk outcomes (Mason, et al., 2009).

1.4.2 Ascorbic Acid (Vitamin C)

The term vitamin C is used as the generic descriptor for all compounds exhibiting qualitatively the biological activity of ascorbic acid. The principal natural compound with vitamin C activity is L-ascorbic acid ($C_6H_8O_6$, MW = 176.1). There are two enantiomeric pairs, namely L- and D-ascorbic acid, and L- and D -isoascorbic acid. D-Ascorbic acid and L-isoascorbic acid are devoid of vitamin C activity and do not occur in nature. D-Isoascorbic acid (also known as erythorbic acid) is also not found in natural products, apart from its occurrence in certain microorganisms. It is, however, a byproduct of biosynthetic vitamin C, produced from glucose by a combined chemical and microbial procedure. L-Ascorbic acid is easily and reversibly oxidized to dehydroascorbic which possesses full vitamin C activity, because it is readily reduced to ascorbic acid in the animal body (Ball, 2005).

Ascorbic acid is an essential nutrient for humans. It plays a role in the synthesis of collagen, hormones and neurotransmitters. Both ascorbic acid's function as an antioxidant and free radical scavenger and epidemiologic studies showing that consumption of generous amounts of vitamin C-rich foods such as fruits and vegetables seems to be associated with a lower risk of various cancers suggest that ascorbic acid may also have cancer-preventive properties (Mangels, et al., 1993).

Vitamin C is most sensitive to destruction when the commodity is subjected to adverse handling and storage conditions. Losses are enhanced by extended storage, higher temperatures, low relative humidity, physical damage, and chilling injury. Ascorbic Acid (AA) is easily oxidized, especially in aqueous solutions, and greatly favored by the presence of oxygen, heavy metal ions, and by alkaline pH and high temperature. L-dehydroascorbic acid can be reduced to AA by reducing agents and also can be irreversibly oxidized to form diketogulonic acid, which has no vitamin C activity. Generally, fruits and vegetables show a gradual decrease in AA content as the storage temperature or duration increases (Lee & Kader, 2000).

Lee & Kader (2000) concluded that selection of the genotype with the highest vitamin C content for a given commodity is a much more important factor than climatic conditions and cultural practices in producing high amounts of vitamin C at harvest. Among pre-harvest factors light intensity and temperature are the most important in determining the final vitamin C content of the commodity. Oxidation can occur in the presence of catalysts, oxidase enzymes, or as a result of heat during processing. Therefore, vitamin C losses continue through postharvest handling, processing, cooking, and storage of fruits and vegetables.

1.4.3 Polyphenols

Recently, researchers have become interested on the importance of polyphenols on the diet, which could be explained for the antioxidant properties in those compounds, and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004),. Another reason is also because phenolics play a role within quality characteristics of plant foods, such as color, as many of them are pigments, such as anthocyanins, also when oxidize enzymatically may origin enzymatic browning, which is undesirable in food processing. Finally, another function of those compounds could be aroma and flavor (Martín-Belloso & Soliva-Fortuny, 2010).

Briefly, a polyphenol structure consists in several hydroxyl groups on aromatic rings. Many plants with a polyphenol structure have been described in higher plants, where several thousands of them were found in edible plants, where are produced as secondary metabolites via the shikimic acid pathway. These compounds may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004).

The most widespread and diverse group of the polyphenols in *Brassica* are the flavonoids. In addition, other phenolic compounds such as benzoic acid or cinnamic acid derivatives have been identified in fruits and vegetables (Podsędek, 2007). Thus, the content and type of polyphenol may vary between cultivars, harvesting stage, cultural practices, or also within different parts of the same crop. Significant levels of hydroxycinnamic acids have also been reported in *B. oleracea* species, like kale, cabbage, broccoli, and cauliflower, where important levels of chlorogenic acids have previously been reported in leafy *Brassica* species (Cartea, Francisco, Soengas, & Velasco, 2011). Specifically, broccoli found to have a high anti-oxidant activity correlated significantly and positively with total phenolics (Moreno, Carvajal, López-Berenguer, & García-Viguera, 2006).

1.4.4 Antioxidants

As stated before, some recent researches indicate that the high consumption of cruciferous vegetables may contribute to prevent degenerative diseases mainly for its antioxidants properties (Singh, Upadhyay, Prasad, Bahadur, & Rai, 2007; Gliszczyńska-Świgło, Ciska, Pawlak-Lemańska, Chmielewski, Borkowski, & Tyrakowska, 2006).

To understand the role of antioxidants in diet, first the concept of free-radicals and antioxidant compound itself must be clarified. A free radical is a molecule that contains an unpaired electron in its outer orbit and that can exist independently. As molecular oxygen is a universal electron acceptor that allows aerobic organisms to use energy stored in foodstuffs, such as carbohydrates, fats, and protein, it also contain two unpaired electrons with parallel spin configurations. Because electrons must have opposite spin to occupy the same orbit, electrons added to molecular oxygen must be transferred one at a time during its reduction, resulting in several highly reactive intermediates. For that reason, is widely accepted and experimentally proven that this catabolic process can generate oxygen free radicals and other reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot -}$), hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2). Under normal physiological conditions, the majority of ROS is produced in the mitochondrial electron transport chain (ETC) since 90% of the oxygen consumption by the body is reduced to water in the mitochondria (Ji, 1999; Clarkson & Thompson, 2000).

In general, to be called antioxidant, a compound must be able to donate an electron and/or hydrogen atom and prevent or delay oxidation of an oxidizable substrate. They can act in different ways: by metal chelation (preventing free radical formation), scavenging free radicals, acting as chain-breakers (stopping propagation of the free radicals), being part of the redox antioxidant network, and regulating gene expression (Packer, 2005).

A number of methods have been developed to measure the efficiency of dietary antioxidants either as pure compounds or in food extracts, as well as to determine the antioxidant activity of plasma as an index of the antioxidant status in vivo. These methods focus on different mechanisms of the antioxidant defense system, i.e., scavenging of oxygen and hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of lipid peroxidation, or chelation of metal ions (Pulido, Bravo, & Saura-Calixto, 2000).

There is a need for quantitative data on the antioxidant content of various subspecies of *Brassica* vegetables. Such information will not only increase the understanding of the function of these antioxidant phytochemicals in lowering incidence of aging and other chronic diseases, but the studies related to qualitative and quantitative distribution of primary antioxidants in cruciferous vegetables may also help in breeding programs to develop new germplasm with a high content of such phytochemicals (Singh, Upadhyay, Prasad, Bahadur, & Rai, 2007).

1.5 Objective

The main objective of this research work was to evaluate the main bioactive compounds, specifically antioxidant capacity compared in two methods (DPPH and FRAP), total polyphenol content and fatty acid quantification, in fresh-cut Bimi® broccoli (*B. oleracea* Italica x Alboglabra) during shelf life, after proper sanitation.

Additionally, this work aimed at the comparison of different sanitizing techniques, such as neutral electrolyzed water (NEW), superatmospheric oxygen (HO_2) MAP and UV-C radiation, applied single and combined, as alternatives to the use of chlorine (NaOCl).

2 MATERIALS AND METHODS

2.1 Plant material

Bimi® broccoli was field grown (*B. oleracea* Italica Group × Alboglabra Group) in the Southeast Mediterranean coast by Campo de Lorca SCL (Murcia, Spain). Immediately after hand-harvesting, the broccoli was forced-air pre-cooled at 1°C and then transported with top icing about 90 km to the Pilot Plant of the Technical University of Cartagena (UPCT), where it was stored in a cold room at 1°C and relative humidity of 95%. The following morning the broccoli was processed (Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011).

Minimal processing was accomplished on the following day in a disinfected cold room at 8°C. Broccoli was carefully inspected, selecting those free from defects and with a similar visual appearance. The sound pieces were cut in approximately 15 cm-long spears, 6-12 mm stalk diameter while leaves were removed with a sharp knife.

2.2 Sanitizing techniques

After minimal processing, the sanitizing techniques applied were:

1. Control: plant material washed for 1 minute in 5°C tap water.
2. Sodium hypochlorite (Chlorine; NaOCl): washing at 5ºC for 2 min with 100 ml l^{-1} NaOCl acidified with citric acid to reach pH= 6.5±0.10. The ratio volume sanitizer:plant was 5L:300g. Subsequently, the produce was rinsed in tap water at 5°C for 1 minute and let drain in a disinfected basket for another minute.
3. Neutral electrolyzed water (NEW): the broccoli was washed at 5ºC with 10 0µl l^{-1} free chlorine neutral electrolyzed water (Envirolyte EL 400, Spain) at pH = 7± 0.1 and ORP of 900 mV. The contact, rinsing and draining time and ratio volume sanitizer:plant was the same described above;
4. UV-C: just before packaging, a 6.0 kJ m^{-2} UV-C dose was applied by using the equipment fully described below.

5. High O_2: Packing in Active Modified Atmosphere (MAP), with a initial partial pressure of 90 kPa;
6. NEW+O_2: a combination of the electrolyzed water and High O_2 treatments above described;
7. NEW+ UV-C: First, a UV-C dose was applied according to procedures described below by following to washing with Neutral Electrolyzed Water;
8. UV-C+O_2: a mixture of UV-C radiation and High O_2 treatments above described
9. MIX: UV-C + NEW + High O_2 combined together in this order.

Fig. 3 – *Washing and rinsing steps in aqueous solutions (Courtesy of GPR).*

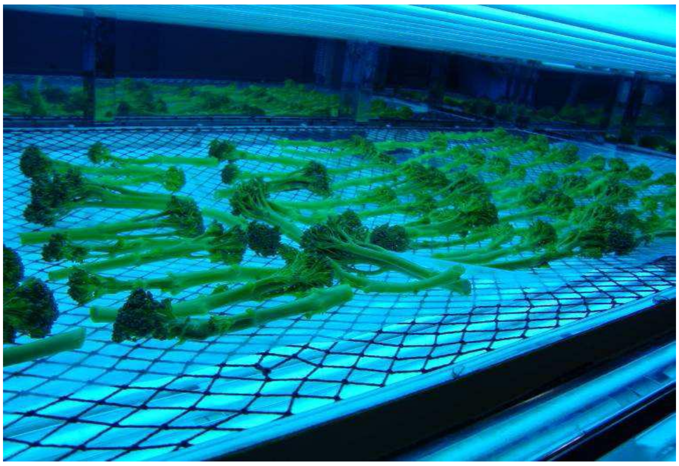

Fig. 4 – *UV-C chamber with Bimi®* (Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011).

2.3 UV-C radiation chamber

The UV-C equipment consisted of two banks of 15 stainless steel reflectors, e ach with unfiltered germicidal emitting lamps (TUV 36W/G36 T8, Philips, Eindhoven, Netherlands). One bank was suspended horizontally over the radiation vessel and the other was placed below it. The samples were placed between the two lines of UV-C lamps at 15 cm above and below over a 35 µm thick bioriented PP film. This film was placed over a steel frame supporting a polystyrene net that minimized blockage of the UV-C radiation. The treatment chamber was covered with a protective reflecting inner layer that enhanced homogeneous distribution of the emitted light and allowed indirect illumination of practically all sides. In order to determine the UV-C radiation intensity of the lamps and to verify the influence on blockage of the polystyrene net, a VLX 254 radiometer (Vilber Lourmat, Marne la Vallée, France) was used. The applied UV-C intensity was calculated as the mean of 18 UV-C readings on each side of the net. Thus both sides received the same UV-C intensity. The UV-C light intensity was kept constant and the applied dose was varied by altering the exposure time at the fixed distance (Artés-Hernández, Escalona, Robles, Martínez-Hernández, & Artés, 2009).

2.4 Experimental Design

For passive modified atmosphere packaging (MAP), samples of about 200g of broccoli per treatment were randomly placed in 1200 ml polypropylene (PP) baskets and thermally sealed on the top with a 50 µm micro perforated bi-oriented PP film (BOPP) (Plásticos del Segura, Murcia, Spain). Five replicates of one basket per treatment and MAP storage duration (processing day and after 5, 9, 15 and 19 days) were prepared and stored in dark cold rooms at 5ºC.

For statistical purposes, the treatments were described in the table below as a complete factorial design, with a level of presence and/or absence. For control, two levels were applied: Chlorine (NaOCl), with absence of the three levels (0, 0, 0), and Control (H_2O), as -1, -1, -1.

Table 2 – *Schematic experimental design for the combined application of NEW, UV light and high O_2.*

Treatment	NEW	UV	O2
Control (H_2O)	-1	-1	-1
Chlorine (NaOCl)	0	0	0
HO_2	0	0	1
NEW	1	0	0
UV-C	0	1	0
NEW+HO_2	1	0	1
NEW+UV-C	1	1	0
UV+HO_2	0	1	1
MIX	1	1	1

2.5 Nutritional Quality

After the minimal processing and packing in Bimi® broccoli, the nutraceutical aspects have been analyzed individually, to verify the influence of the treatments above described on those compounds throughout storage time.

Before the nutritional quality assays, also intended for tissue preparation, ground broccoli samples were frozen in liquid N_2, and subsequently grinded with an electric mill (IKA, A11 basic, Berlin, Germany) at 12,700 × g for 10s, where subsequently stored at −80°C in darkness.

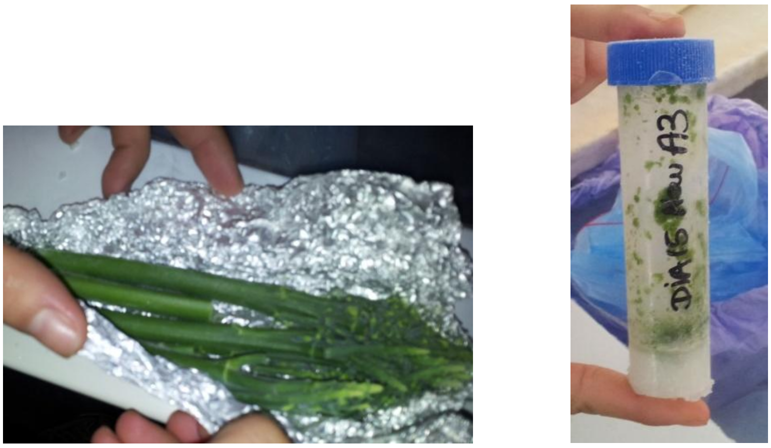

Fig. 5 – *Frozen Bimi® Broccoli - Whole and Grinded (Courtesy of GPR).*

2.5.1 Fatty Acid Content

All procedures were carried in faint light at 4°C using chilled solvents containing BHT (0.01% [w/v]) supplied by Merck Farma y Química, S.A. (Madrid, Spain) and glassware. The organic solvents: methanol (HPLC grade), acetic acid, hexane and 2-propanol (residue analysis) were all purchased by Panreac Química S.A. (Barcelona, Spain) as well as the non-organic solvent used, sulfuric acid. Chloroform was supplied by Fischer Scientific (Madrid, Spain). Ultrapure water type 1 (Milli-Q®) was obtained using a Milli-Q system (Academic Gradient A10, Millipak™ 40, Millipore, Paris, France).

The fatty acid was extracted according the method described by Page, Griffiths, & Buchanan-Wollaston (2001), with some modifications. First, approximately 0.2 g fresh weight of plant sample was placed in glass (Pyrex®, England, UK) culture tubes. Subsequently, on each tube 1 ml acetic acid 0.15M and chloroform/methanol (1:2 v/v; 7.5 ml) was added. After being homogenized on an orbital shaker (Model 4535, Forma Scientific, USA), 2.5 ml ultrapure water was adjoined. Phase separation was facilitated by low-speed centrifugation; the lower chloroform ($CHCl_3$) phase containing the lipids was removed and dispensed into another 15 ml Pyrex® (England, UK) tubes protected from light. Then, the samples were evaporated under gaseous nitrogen by a TurboLV Evaporator (Caliper Life Sciences, Massachusetts, USA).

Lipids were quantified as their fatty acid methyl ester derivatives obtained by transmethylation performed in 2.5% (v/v) sulfuric acid in anhydrous methanol (2 ml). Next, the tubes were put in 1-hour 90°C water bath followed by a quick cold water bath.

Afterwards, 2 ml n-hexane was added to extract the fatty acids followed by 2 ml ultrapure water. The superior phase with n-hexane containing the methyl fatty acids was recovered and put into amber vials (Hewlett-Packard, California, USA).

The samples were analyzed by gas chromatography (GC)/ mass spectrometry (MS) in an Agilent 6890N Network Gas Chromatographer system (Agilent Technologies, Waldbronn, Germany) consisting of a turbo pump, an autosampler and a temperature-controlled column coupled to a sensitive array detector equipped with a GC-ICP-MS interface (Tarrazona-Díaz, Viegas, Moldão-Martins, & Aguayo, 2011). An Agilent DB-23 capillary column (60 m × 0.25 mm × 0.25 µm i.d.; Agilent, Darmstadt, Germany) was used. For standards, Palmitic, Linolenic and Stearic acids were all purchased by Sigma-Aldrich Química (Madrid, Spain).

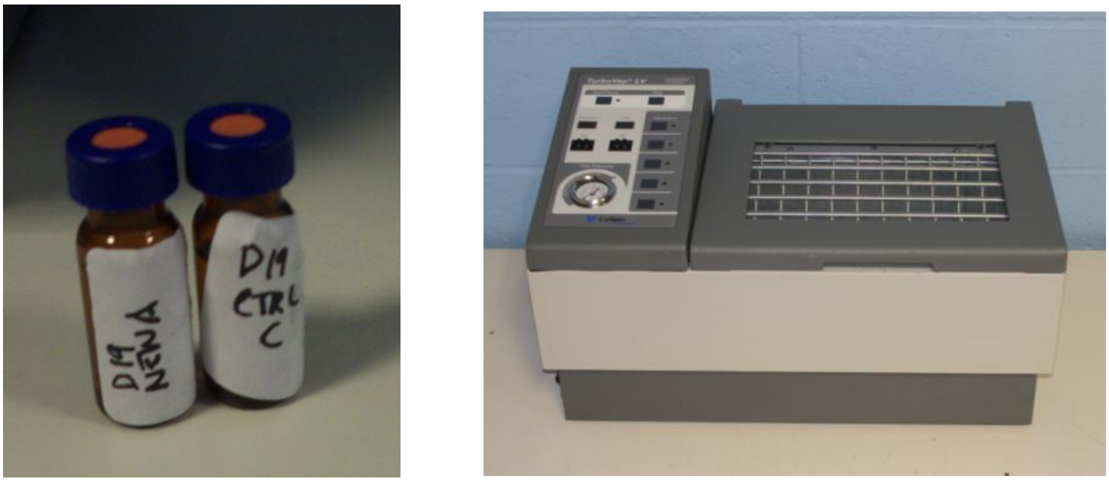

Fig. 6 – *Amber vials with fatty acid sample and Turbovap® LV Evaporator (Courtesy of GPR).*

2.5.2 Ascorbic Acid (Vitamin C)

Firstly a buffer was made using methanolic water (5:95 v/v) with sodium fluoride (NaF, Panreac Barcelona, Spain) added with 0.1M Citric acid (Sigma-Aldrich, Spain) and 0.05% EDTA (Ethylenediamine tetraacetic acid, Sigma-Aldrich, Spain). In a light-protected Falcon tube 5g of previously grinned frozen broccoli was added with 10 ml of cold buffer, and subsequently crushed on a stirrer (T10 Ultra Turrax, IKA Equipments, Germany).

Initially, cheesecloth was used for first filtering in falcon tubes, and the pH was adjusted to 2.35 with a help of hydrochloric acid 6N (Panreac Barcelona, Spain). Afterward, the filtered samples were distributed in 2 ml eppendorf tubes for centrifugation at 10500G for

2 minutes in cold (2-5ºC). The second filtering was performed using 0.45 µm Sep-Pack with C18 syringe (Waters, Milford, US) into amber vials (750µL). The samples were analyzed by chromatography in a HPLC, in a reverse phase column (Gemini NX C18, Phenomenex, France). The standards used (Ascorbic and Dehydroascorbic acids) were all supplied by Sigma-Aldrich Spain.

2.5.3 Sample preparation for total polyphenol content and antioxidant capacity (FRAP and DPPH) assays

The extraction was made using 0.5g fresh weight of frozen grinned broccoli and 3 ml methanol HPLC-grade (Panreac Barcelona, Spain) into a 30 ml tubes placed inside of a poliestirene box with ice, followed by 1-hour agitation in an orbital shaker (Stuart Orbital Shaker SSL1, Bibby Scientific Limited, Staffordshire, UK) at 200 rpm. Subsequently, the samples were taken from the tubes with pasteur pipettes and placed in 2 ml eppendorfs tubes (Eppendorf Ibérica, Madrid, Spain), to be centrifuged at 10500G for 10 minutes in a 4ºC centrifugue (Heraeus Fresco 21, Controltécnica, Spain).

2.5.3.1 Total polyphenol content

The total polypenol content analysis was adapted from Swain & Hillis (1959) method. Firstly, a Folin reagent 1N was prepared using 1:1 v/v Folin-Ciocalteu 2N reagent (Sigma-Aldrich, Spain) and the same amount of ultrapure (Milli-Q®) water (Academic Gradient A10, Millipak™ 40, Millipore, Paris, France). Then, a mix buffer was made utilizing 0.4% Sodium hydroxide (Panreac, Barcelona, Spain) and 2% Sodium carbonate (Panreac, Barcelona, Spain) over Milli-Q® water.

Concisely, on each well of a 96-well poliestirene microplate (Grenier Bio-One Ltd., Stonehouse, UK) over an ice bed, 19.2µl sample extract was placed among 29µl Folin 1N (1:1 v/v). After waiting 3 minutes, 192µl mix buffer was added, in triplicate. The microplate was allowed 1 hour to react at room temperature and darkness. Subsequently, the absorbance was measured at 750 nm by using a Multiscan plate reader (Tecan Infininte

M200, Männedorf, Switzerland). The results were expressed in Chlorogenic Acid Equivalent per kg^{-1} fw.

For standard curve, chlorogenic acid (Sigma-Aldrich, Madrid, Spain) was used over the dilutions of 0, 0.024, 0.05, 0.076, 0.1, 0.2, 0.3 and 0.4 mg/ml. The blank was prepared employing 19.2µl of methanol instead sample extract in the same method described above.

2.5.3.2 Antioxidant capacity (FRAP assay)

The total antioxidant capacity was determined by the ferric reducing ability of plasma (FRAP) assay, described by Benzie & Strain (1996), with some modifications. All used reagents were of analytical spectrophotometric grade. The TPTZ (2,4,6-tripyridy-s-triazine) and Ascorbic Acid were supplied by Sigma-Aldrich Spain. Instead, the ferric chloride, hydrochloric acid (HCl), glacial acetic acid and sodium acetate trihydrate (for acetate buffer) were provided by Panreac Química S.A. (Barcelona, Spain).

Initially, a FRAP reagent was prepared using 2.5 ml of TPTZ solution (10 mM) in 40 mM HCl plus 2.5 ml of ferric chloride ($FeCl_3$ $6H_2O$) and 25 ml acetate buffer (300 mM, pH=3.6). Previously, the FRAP reagent was prepared freshly and warmed at 37ºC for two hours. After that, on each well of a 96-well poliestirene microplate (Grenier Bio-One Ltd., Stonehouse, UK) over an ice bed, 6µl of plant extract previously described was mixed with 198µl of FRAP reagent, in triplicate. The absorbance of samples was measured at 593 nm after 30 min by using a Multiscan plate reader (Tecan Infininte M200, Männedorf, Switzerland). Results were expressed as mg ascorbic acid equivalent antioxidant capacity (AAE) per kg^{-1} fw.

Aqueous solutions of known ascorbic acid concentrations in the range of 0.04 - 0.42mg/ml were used for calibration, in triplicate. For blanks, ultrapure water was used instead, in the same method described above.

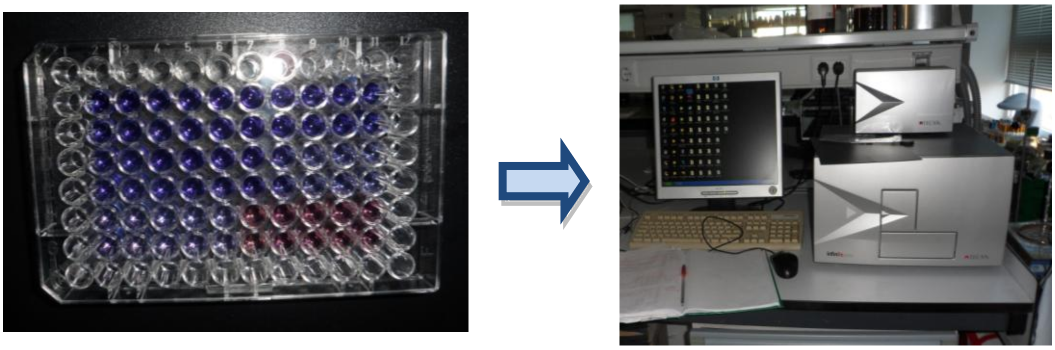

Fig. 7 – *Scheme of microplate containing DPPH reagent following Multiscan plate reader (Courtesy of GPR).*

2.5.3.3 Antioxidant capacity (DPPH assay)

For DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, the method described by Brand-Williams, Cuvelier, & Berset (1995) was adopted with some modifications. All the procedures were carried out in dim light. Briefly, a methanolic stock solution of DPPH (Sigma-Aldrich Química S.A., Madrid, Spain) was prepared with initial concentration of 0.24 g/l, and kept cold at darkness. Since DPPH stock solution is too concentrated, the dilution should have at 593nn, a value of 1.1±0.2. For that reason, a dilution a 300µl DPPH stock + 700µl MeOH (Panreac Química, Barcelona, Spain) was prepared every day.

On each well of a 96-well poliestirene microplate (Grenier Bio-One Ltd., Stonehouse, UK) over an ice bed, 21µl sample extract previously described plus 194µl DPPH everyday solution were combined. The covered microplate was allowed to react in the dark at room temperature. Subsequently, the absorbance of samples was measured at 517 nm after 30 min by using a Multiscan plate reader (Tecan Infininte M200, Männedorf, Switzerland). Results were expressed as mg ascorbic acid equivalent (AAE) per kg^{-1} fw.

Regarding the standard curve, ascorbic acid (Sigma-Aldrich Química, Madrid, Spain) was used with concentrations ranging from 3.91 to 62.5 µg/ml. For blanks 21µl of methanol was employed instead sample extract.

2.6 Statistical Analysis

Results were analyzed with a two factor ANOVA, including the effect of time and type of treatment. When differences were identified, multiple comparison Tukey test was used.

Additionally, comparison of results at the last day of storage (19 days) was performed by means of a non-parametric Kruskal-Wallis test for k independent samples. All test were performed at a 95 % confidence level using IBM SPSS for Windows v.20 (IBM, New York, USA).

3 RESULTS AND DISCUSSION

3.1 Fatty Acid Content

Regarding the lipid content, the three main fatty acids in Bimi® broccoli were quantified as stearic (18:0), linolenic (18:3) and palmitic (16:0) acids, expressed in µmol g^{-1} fw.

3.1.1 Palmitic Acid

In accordance with previous reports in broccoli, palmitic acid was the principal saturated fatty acid, also in Bimi®, in comparison to stearic acid (Murcia, López-Ayerra, & García-Carmona, 1999). Sanitizing techniques initialized palimitic acid content of 3.4 µmol g^{-1} fw, but presented a higher decrease in 5 days, with 1.4 µmol g^{-1} fw on 19th day of storage. Conversely, chlorine showed stable or lightly increasing values upfront 5th day, with 2.3 µmol g^{-1} fw on the 9th day.

NEW treatment responded with initial content of 2.23 µmol g^{-1} fw, in opposition, presented a decrease of 10% on its content throughout storage, with all other treatments, remaining constant. Nevertheles, NEW combined with superatmospheric MAP (NEW+HO_2) had slightly increasing values, starting with 2.5 and responding with 2.8 µmol g^{-1} fw during 19 days.

UV-C ionized broccoli started with 2.7 µmol g^{-1} fw on processing day, yet steadily decreases with losses of around 10-12% throughout storage. A similar trend was also observed in samples combined with superatmospheric oxygen MAP (UV-C+HO_2), initiating with 2.8 µmol g^{-1} fw, but 2.50 µmol g^{-1} fw on 19th day of storage time.

On the other hand, when single packed with high oxygen (HO_2), Bimi® started with 2.85 µmol g^{-1} fw, thus could maintain the palmitic acid content with no significant diminution during storage time, in discordance with Zhuang, Hildebrand, & Barth (1995), which found great losses in broccoli buds during MAP at low temperatures.

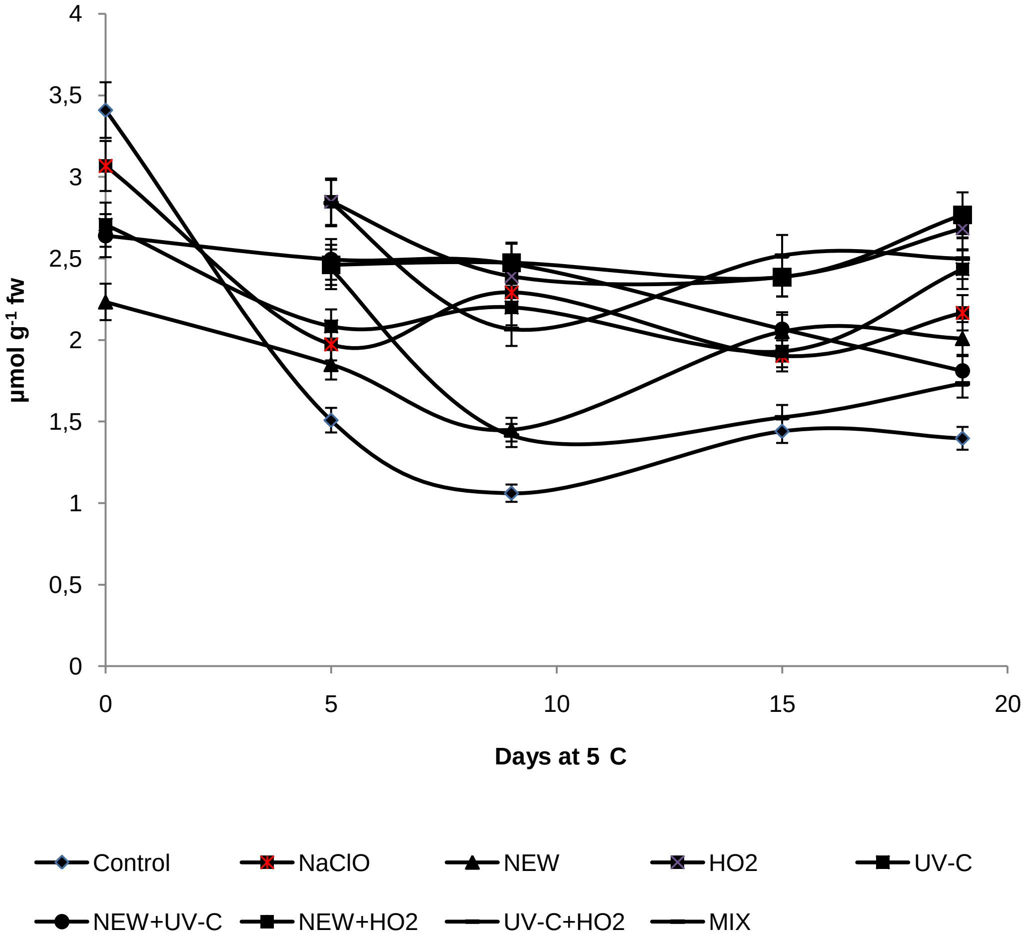

Fig. 8 – *Palmitic acid (16:0) content changes in Bimi® broccoli alternatively sanitized and stored at 5°C up to 19 days. Data represent means of three replicates (±SE).*

Performing a two-way ANOVA, could be verified the influence of sanitizers over storage time with 95% of confidence ($F(28,82)=5.2$). Also, storage time and sanitizing techniques individually can contribute for changes in palmitic acid content in Bimi®. However, to compare within treatments, applied single and combined, after 19 days, a Mann-Whitney test was subjected (see 3.5).

Table 3 - *Two-way factorial ANOVA performed by an independent factorial design with 95% of confidence level of palmitic acid content in Bimi® broccoli during storage time.*

Tests of Between-Subjects Effects					
Dependent Variable: Palmitic Acid (umol/g fw)					
Source	**Type IV Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	32,106[a]	40	,803	12,828	,000
Intercept	598,285	1	598,285	9561,697	,000

storage	10,507[b]	4	2,627	41,978	,000
treatm	15,565[b]	8	1,946	31,095	,000
storage * treatm	9,061	28	,324	5,172	,000
Error	5,131	82	,063		
Total	635,522	123			
Corrected Total	37,237	122			
a. R Squared = ,862 (Adjusted R Squared = ,795)					
b. The Type IV testable hypothesis is not unique.					

3.1.2 Stearic Acid

On processing day, control samples exhibited 0.5 µmol g^{-1} fw, with a reduction of 50% throughout storage time. On the other hand, Bimi® washed with chlorine presented stable values until 9th day of storage, however decreased its content whereof the 15th day. Regarding NEW, the samples showed stable or lightly increasing values, starting at 0.5 µmol g^{-1} fw on the processing day.

For combined techniques, NEW+UV-C presented 0.49 µmol g^{-1} fw, but steadily decreased its content up to 0.2 µmol g^{-1} fw through 19 days of storage. Moreover when combined with HO_2, NEW revealed stable values ranging from 0.5 up to 0.47 µmol g^{-1} fw in 19 days. Also, UV-C+HO_2 demonstrated similar behavior with no decrease of stearic acid during storage time.

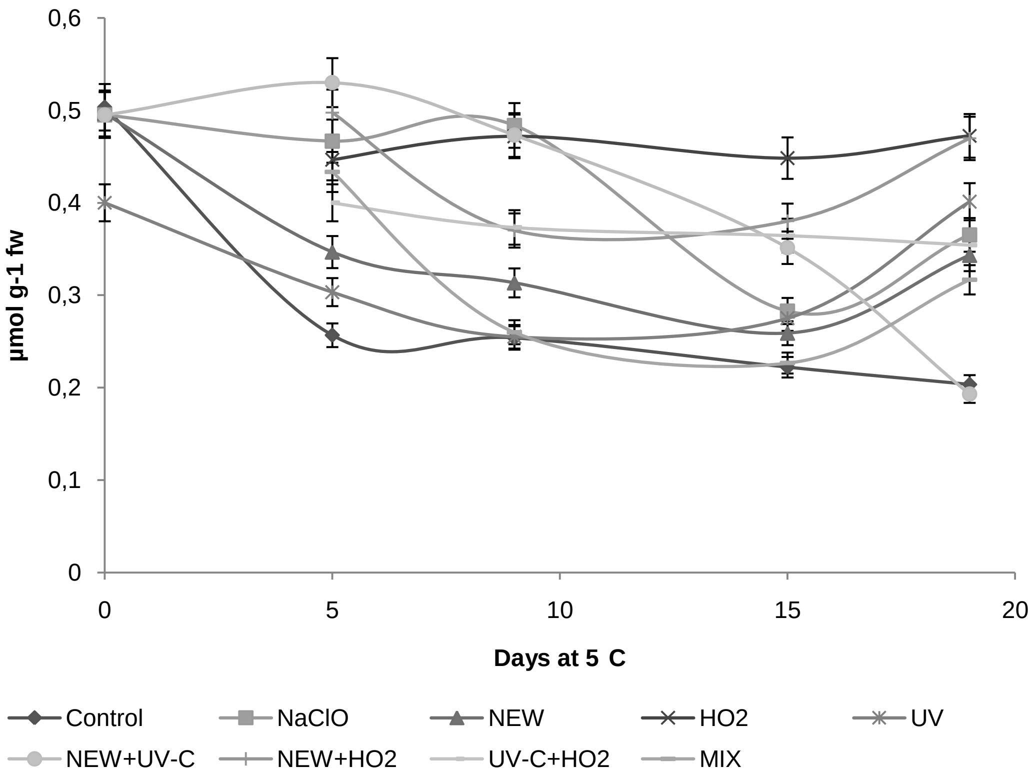

Fig. 9 – *Stearic acid (18:0) changes in Bimi® broccoli during storage time at 5°C after several sanitizing treatments expressed in µmol g^{-1} fw. Data represent means of three replicates (±SE).*

The sanitizing techniques can influence the stearic acid content in Bimi® through 19 days of storage time ($F(28,82)=10.8$). A two-way analysis shows that storage time and treatment, as fixed factors, are significant with an alpha level less than .05.

Table 4 - *Two-way factorial ANOVA performed by an independent factorial design with 95% of confidence level of stearic acid content in Bimi® broccoli during storage time.*

Tests of Between-Subjects Effects					
Dependent Variable: Stearic Acid (umol/g fw)					
Source	**Type IV Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	1,259[a]	40	,031	24,761	,000
Intercept	17,421	1	17,421	13700,538	,000
storage	,506[b]	4	,127	99,550	,000
treatm	,481[b]	8	,060	47,262	,000
storage * treatm	,385	28	,014	10,823	,000
Error	,104	82	,001		
Total	18,784	123			
Corrected Total	1,364	122			
a. R Squared = ,924 (Adjusted R Squared = ,886)					
b. The Type IV testable hypothesis is not unique.					

3.1.3 Linolenic Acid

Linolenic acid was also the principal fatty acid in Bimi®, corroborating the same reported in broccoli (Murcia, López-Ayerra, & García-Carmona, 1999). Control samples showed 8.43 µmol g^{-1} fw of linolenic acid on the processing day, but showed the highest decrease on its content during shelf-life, mainly caused by lipid peroxidation. Analogously, NEW presented higher decreases, with losses of around 65%, and also could be explained by oxidizing properties of electrolyzed water enhancing lipid peroxidation. On the other hand, samples washed with NaOCl steadily decreased their linolenic acid content during storage, with 3.82 µmol g^{-1} fw at 15th day.

With UV-C, the initial content was 5.50 µmol g^{-1} fw, yet an increase was found during 15 days of storage. Nevertheless, presented the lowest decrease over storage, as well as with superatmospheric O_2 MAP. Nevertheless, UV-C combined with NEW found a decline starting from 15 days.

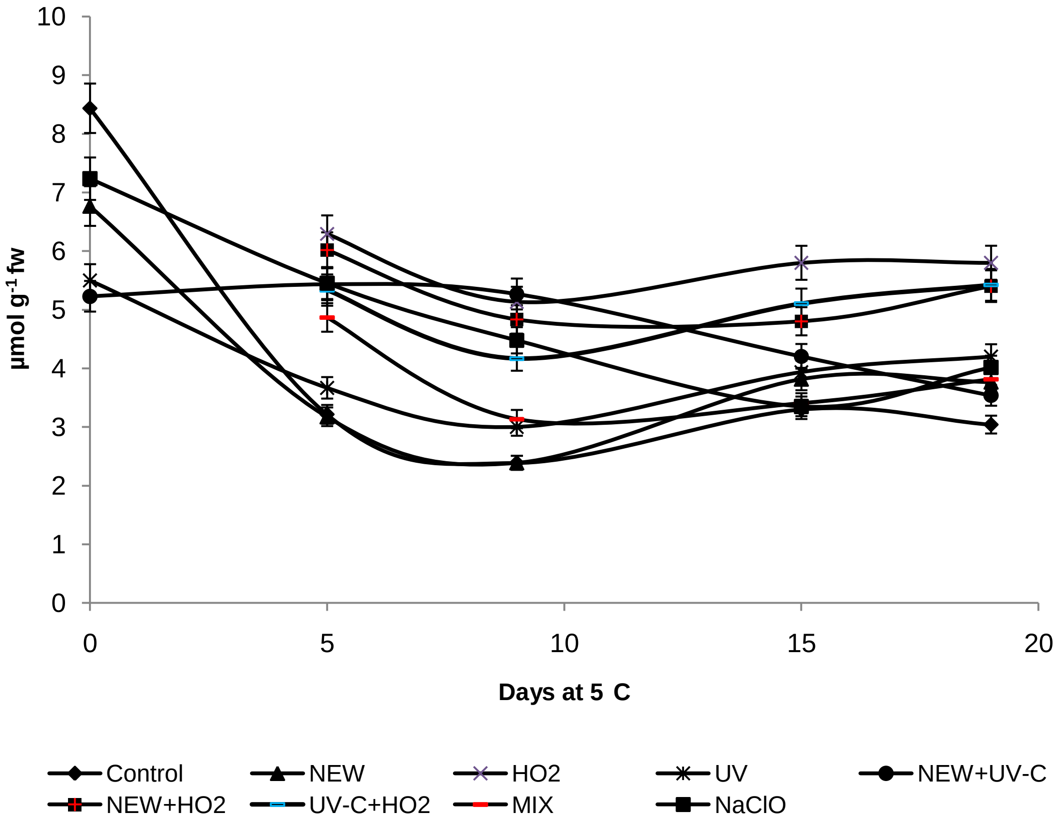

Fig. 10 – *Linolenic acid (18:3) content changes in Bimi® broccoli alternatively sanitized and stored at 5°C up to 19 days. Data represent means of three replicates (±SE).*

Superatmospheric O_2 MAP has proved to be effective for maintaining linolenic acid content with initial values of 6.29 µmol g^{-1} fw, as before mentioned, the lowest decrease over storage. Also, the same trend was found when combined with UV-C (UV-C+HO_2), with 5.42 µmol g^{-1} fw during 19 days of storage, and when mixed with NEW (NEW+HO_2), with no significant differences between them ($p>0.05$).

Finally, the losses in fatty acids might be favored by fractionation of lipidic compounds since in the micellar structure of the fat, the phospholipids (the most polar lipids) surround the triglyceride spheres and act as a physical barrier against the oxygen. They decrease in quantity during hydrolysis because of their susceptibility to oxidation observed in an overall decline in phospholipids during ripening in vegetables (Murcia, López-Ayerra, & García-Carmona, 1999).

The table below shows the influence of sanitizing treatments, regarding linolenic acid content in Bimi® broccoli, through storage time, with 95% of confidence ($F(28,82)=24.7$). The two fixed factors, Storage Time, $F(4,28)= 390.6$, and Treatment $F(8,28)= 141.7$, were also significant in an alpha level of .05 for all statistical tests.

Table 5 - *Two-way factorial ANOVA performed by an independent factorial design with 95% of confidence level of linolenic acid content in Bimi® broccoli during storage time.*

Tests of Between-Subjects Effects					
Dependent Variable: Linolenic Acid (umol/g fw)					
Source	**Type IV Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	229,722[a]	40	5,743	77,444	,000
Intercept	2637,077	1	2637,077	35560,482	,000
storage	115,860[b]	4	28,965	390,589	,000
treatm	84,044[b]	8	10,505	141,664	,000
storage * treatm	51,214	28	1,829	24,665	,000
Error	6,081	82	,074		
Total	2872,880	123			
Corrected Total	235,802	122			
a. R Squared = ,974 (Adjusted R Squared = ,962)					
b. The Type IV testable hypothesis is not unique.					

3.2 Ascorbic Acid (Vitamin C)

Due to an experimental error, the results of vitamin C assay could not be measured.

3.3 Total Polyphenol Content

On processing day, the washing with chlorine (NaOCl) reduced the initial polyphenol content (1357.3 mg ChAE kg^{-1} fw) in approximately 12-14% on its content, without significant differences between treatments. NEW and UV-C presented a slightly increase on polyphenol content during five days of storage (1241.8 y 1441.6 mg ChAE kg^{-1} fw, respectively). Conversely, the combined technique with superatmospheric O_2 MAP, presented an increase during 19 days of storage time.

The triple combined treatments (UV-C+HO_2+NEW – MIX) presented the highest increase in 9 days, with 1547.3 mg ChAE kg^{-1} fw throughout storage time. Likewise, the UV-C+HO_2 presented the same behavior during 15 days of storage, however has decreased its content on 19th of storage in around 20%. Nonetheless, while studying another *Brassica*, Tomás-Callejas, Otón, Robles, Artés, & Artés-Hernández, (2012), proved that phenolic content was kept in minimal processing enriched-O_2 MAP with UV-C during 11 days of storage.

In different concentrations of UV-C ionized Bimi®, Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández (2011), also found an increase on total polyphenol content during the ending of storage time. In this work, UV-C showed a high increase on its content since 9 days, remaining 1584.3 mg ChAE kg^{-1} fw throughout 19 days of storage time. This fact could be verified because UV irradiation induces the accumulation of phenolic compounds and flavonoids in plants as a defense mechanism against irradiation. Thus, the increase in total phenolics can also be attributed to the phenylalanine ammonia-lyase activity (PAL), which is one of the key enzymes in the synthesis of phenolic compounds in plant tissues (Alothman, Bhat, & Karim, 2009).

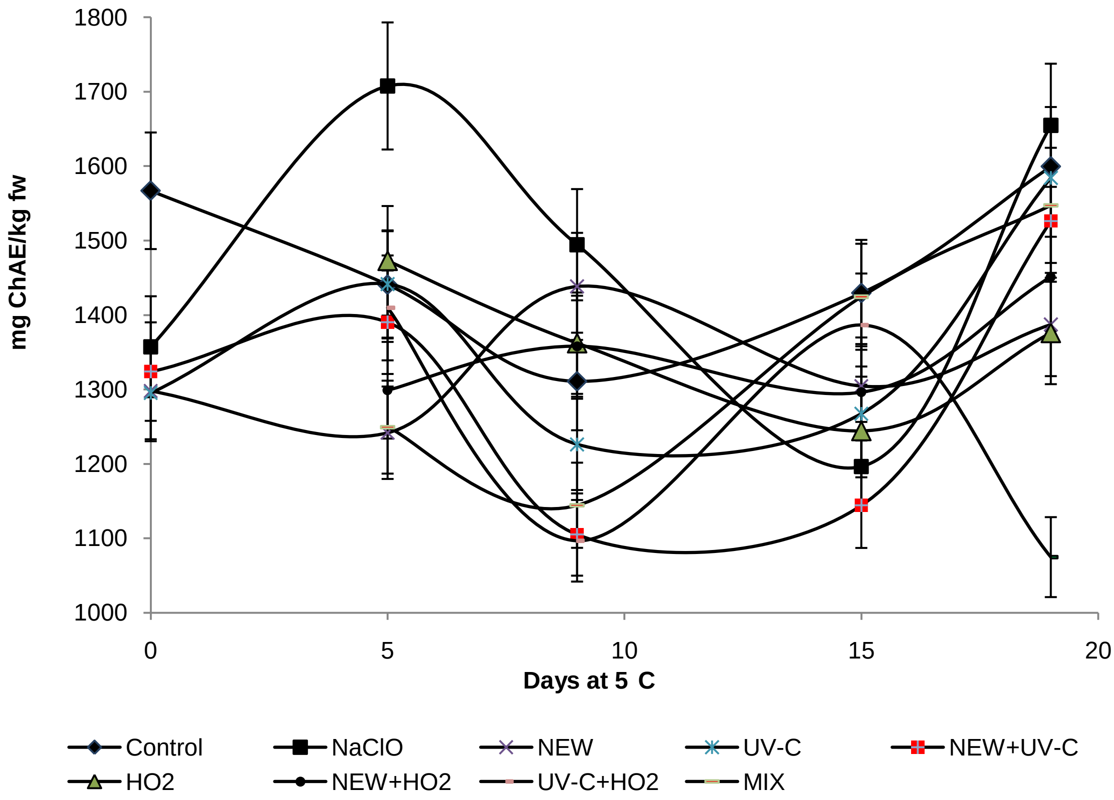

Fig. 11 – *Total phenolics changes in Bimi ® broccoli with different sanitizing methods, during 19 days stored at 5°C. Data represent means of three replicates (±SE).*

Regarding NEW, the maximum polyphenol content was observed on day 9 (1438.5 mg ChAE kg^{-1} fw), thus, a decrease was found registering during 19 days with 1387.3 mg ChAE kg^{-1} fw, in opposite trend verified with other treatments. This same behavior also was encountered in mizuna baby leaves (*B. rapa* Japonica) by Tomás-Callejas, Martínez-Hernández, Artés, & Artés-Hernández (2011). Nevertheless was not validated in other broccoli cultivars (Vandekinderen, et al., 2009).

On the other hand, all treatments presented a reduction of 15% on its content after 9 days of shelf life yet all treatments registered an increase on polyphenols content throughout all days of conservation. Also should be mentioned that antioxidants are not only phenolic based, and other compounds such as selenium and tocopherol, could influence to the antioxidant capacity of plant tissues (Tomás-Callejas, Otón, Robles, Artés, & Artés-Hernández, 2012).

Table 6 - *Two-way factorial ANOVA performed by an independent factorial design with 95% of confidence level of polyphenol content in Bimi® broccoli.*

Tests of Between-Subjects Effects					
Dependent Variable: PP ChAE (mg ChAc/kg fw)					
Source	**Type IV Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	8265901,051[a]	40	206647,526	6,145	,000
Intercept	686742424,558	1	686742424,558	20420,171	,000
treatm	1798488,289[b]	8	224811,036	6,685	,000
storage	2069602,056[b]	4	517400,514	15,385	,000
treatm * storage	4439371,146	28	158548,969	4,714	,000
Error	11030834,108	328	33630,592		
Total	706039159,717	369			
Corrected Total	19296735,159	368			
a. R Squared = ,428 (Adjusted R Squared = ,359)					
b. The Type IV testable hypothesis is not unique.					

As reported before a significant interaction between Storage Time and Santizing Treatment, $F(28,328)= 4.7$, was found with 95% of confidence, where the main simple effects of Storage and Treatment were also significant ($F(4,328)=15.4$; $F(8,328)=6.7$, respectively). As before mentioned, treatments could themselves be compared after 19 days using a non-parametric Mann-Whitney test.

3.4 Antioxidant Capacity

3.4.1 Ferric-Reducing Ability of Plasma (FRAP) Assay

Sanitizing treatments reduced the initial total antioxidant capacity of 722.3 mg AAE kg^{-1} fw recording NEW+UV-C and UV-C the lowest values with reductions of approximately 57% on the processing day. Chlorine responded with the maximum total antioxidant capacity on the 5^{th} and 9^{th} but after 15 days of storage, has lowered levels. All treatments with processing day, reduced the total amount of antioxidant capacity after 5 days at 5ºC, but subsequently, an increased was found after 9 days at 5ºC. As expected, the total antioxidant capacity decreased through 19 days of storage time. Thus, in comparison to 15^{th} day, all sanitizing techniques increased the total antioxidant activity.

The UV-C registered its higher value on 9th day (515.3 mg AAE kg^{-1} fw) but steadily decreased through the following storage days, where could be explained to the UV-C damage (Artés-Hernández, Escalona, Robles, Martínez-Hernández, & Artés, 2009). On the processing day, Control showed 722.3 mg AAE kg^{-1} fw, nonetheless presented the higher decrease of 56% after 15 days.

Neutral electrolyzed water (NEW) gradually enhanced the antioxidant capacity during storage, having the maximum after 19 days at 5ºC (531.2 mg AAE kg^{-1} fw). Moreover, while studying Tatsoi (*Brassica rapa* cv rosularis) and Red Chard (*Beta vulgaris* cv flavescens) treated with NEW, Aguayo, Boluda, Le Lann, & Artés (2008) verified a slight oxidant tendency during 9 days at 5°C, where reduced their nutritional rate. For that reason, more studies should be carried to verify the influence of electrolyzed water as sanitizing technique on nutritional values in food commodities. On the other hand, NEW+UV-C showed constant antioxidant capacity behaviour during the refrigerated storage time.

For respiration purposes, the techniques with high oxygen could not be measured on the processing day, instead starting at the 5th day of storage time. UV+HO_2 responded with the maximum total antioxidant capacity (737.8 mg AAE kg^{-1} fw) after 5 days of storage, nevertheless presented a higher decrease, in around 48%, on its content (353.2 mg AAE kg^{-1} fw) throughout 19 days. On the other hand, HO_2 registered initial antioxidant activity of 495.4 mg AAE kg^{-1} fw, yet showed the lowest reduction after 19 days (326.2 mg AAE kg^{-1} fw).

Meanwhile, NEW+HO recorded 307.8 mg AAE kg^{-1} fw through five days of storage, showing an increase at 9 days and subsequently at 19 days of storage, represented as 430.4 mg AAE kg^{-1} fw. Inversely, MIX decreased its content during 9 days of storage (298.1 mg AAE kg^{-1} fw), however an increase was shown during 19 days at 5°C (388.9 mg AAE kg^{-1} fw).

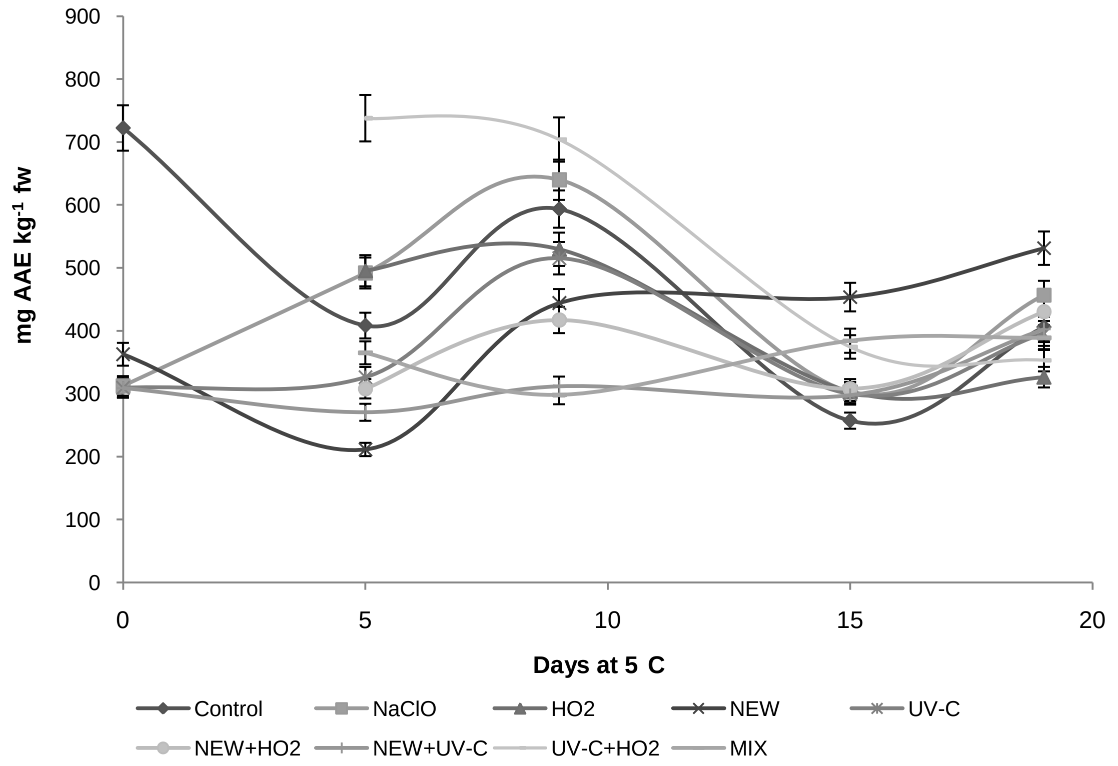

Fig. 12– *Total antioxidant capacity changes (FRAP method) of fresh-cut Bimi® broccoli treated with several sanitizing techniques and stored up to 19 days at 5°C. Data represent means of three replicates (±SE).*

An alpha level of .05 for all statistical tests was used. The main effect of Storage Time was significant F (4,328) = 116.6, as was the main effect of Treatment F (8,328) = 64.8. Finally, could be concluded with 95% of confidence, that there was a significant interaction between the effects of storage time and sanitizing treatment on antioxidant retention, F (24, 288) = 50.2, p<0.01.

Table 7 – *Two-way factorial ANOVA performed by an independent factorial design with 95% of confidence level of antioxidant capacity by FRAP method.*

Tests of Between-Subjects Effects					
Dependent Variable: FRAP AAE (mg AA/kg fw)					
Source	**Type IV Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	5983313,144[a]	40	149582,829	62,017	,000
Intercept	61607832,711	1	61607832,711	25542,613	,000
treatm	1250749,707[b]	8	156343,713	64,820	,000
storage	1125323,794[b]	4	281330,948	116,640	,000
treatm * storage	3392300,879	28	121153,603	50,230	,000
Error	791123,791	328	2411,963		

Total	68382269,646	369			
Corrected Total	6774436,935	368			
a. R Squared = ,883 (Adjusted R Squared = ,869)					
b. The Type IV testable hypothesis is not unique.					

3.4.2 DPPH Free-Radical Scavenging Activity Assay

Regarding DPPH assay, it could be verified the influence of sanitizing techniques over the antioxidant retention. The initial total antioxidant capacity content was similar over the treatments, ranging from 156.9 to 205.8 mg AAE kg^{-1} fw. Both control samples (washed with water and chlorine) showed parallel trends in keeping up antioxidant power, with losses of around 77% during storage time.

NEW responded with the maximum antioxidant capacity on processing day (205.8 mg AAE kg^{-1} fw), and along NEW+UV-C showed the highest decrease throughout storage time, without significant differences between them (7.7 and 0 mg AAE kg^{-1} fw, respectively). While studying mizuna baby leaves treated with NEW, (Tomás-Callejas, Martínez-Hernández, Artés, & Artés-Hernández, 2011) and others concluded that after 11 days at 5°C, a general reduction of 30-40% of the initial total antioxidant activity was found. This fact might be due to vitamin C and carotenoid losses during storage time but further studies down this path should be conducted. Also, in the same study, NEW has been proved less aggressive for maintaining total antioxidant capacity over other forms of electrolyzed water.

Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández (2011) verified that Bimi® treated with UV-C doses increased the antioxidant power over the storage time. However, in this experiment, with a 6.0 kJ m^{-2} UV-C dose, the samples could maintain the antioxidant power during 9 days of storage, but highly decreased through 15 and subsequently 19 days (24.1 and 9.4 mg AAE kg^{-1} fw). For that reason, further researches should be carried to prove the UV-C influence over antioxidant capacity in Bimi® broccoli.

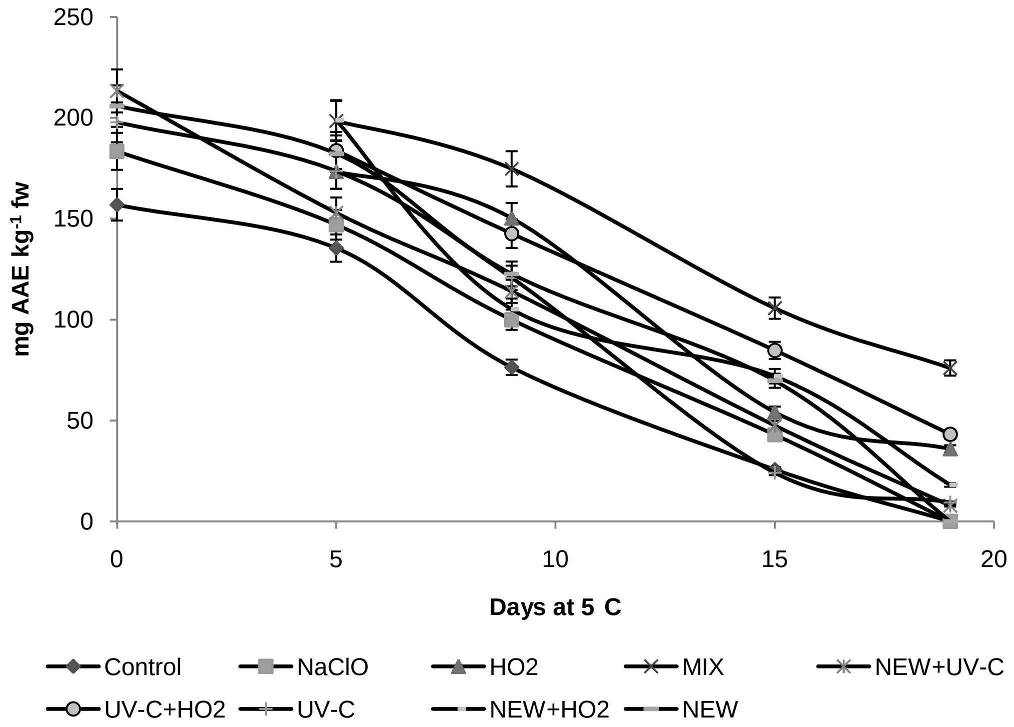

Fig. 13 – *Total antioxidant capacity changes (DPPH method) of fresh-cut Bimi® broccoli treated with several sanitizing techniques and stored up to 19 days at 5°C. Data represent means of three replicates (±SE).*

Regarding samples in superatmospheric oxygen MAP, both single treatment and combined with Neutral Electrolyzed Water (NEW) showed similar trend on the matter of keeping the antioxidant power, with losses in around 85% during storage time. However, the samples combining High O_2 MAP and UV-C radiation (UV-C+HO_2) demonstrated slightly increasing values in comparison of the treatments described above, with 43.1 mg AAE kg^{-1} fw during storage time.

Table 8 - *Two-way factorial ANOVA performed by an independent factorial design with 95% of confidence level of antioxidant capacity by DPPH method.*

Tests of Between-Subjects Effects					
Dependent Variable: DPPH AAE (mg AA/kg fw)					
Source	**Type IV Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	1683140,439[a]	40	42078,511	449,827	,000
Intercept	4108096,388	1	4108096,388	43916,323	,000
treatm	161122,922[b]	8	20140,365	215,304	,000
storage	1467012,752[b]	4	366753,188	3920,661	,000
treatm * storage	42635,707	28	1522,704	16,278	,000
Error	30682,342	328	93,544		
Total	5821919,169	369			
Corrected Total	1713822,780	368			

a. R Squared = ,982 (Adjusted R Squared = ,980)
b. The Type IV testable hypothesis is not unique.

An alpha level of .05 for all statistical tests was used. The main effect of Storage Time was significant, $F(4,328) = 3920.7$, as was the main effect of Treatment, $F(8,328) = 215.3$. Finally, could be concluded with 95% of confidence, that there was a significant interaction between the effects of storage time and sanitizing treatment on antioxidant retention by DPPH method, $F(28, 328) = 16.3$, $p<0.01$.

3.5 Overall results at end of storage

Another aim of this research was to compare within santizing treatments applied single and combined, on each essay studied independently, to determine which sanitizer can retain the best nutritional attributes after 19 days of storage.

For that reason, a non-parametric Mann-Whitney test for independent samples at 95% confidence level was performed to compare the influence of UV-C radiation, NEW and HO_2 MAP applied single and combined, on the 19 days of storage at 5°C, as well as Chlorine (NaOCl) and tap water, shown below.

Table 9 – *Mean values and standard deviation for all determinations made at 19 days of storage. Data represent mean of three replicates.*

	FRAP AAE (mg AA/kg fw)		DPPH AAE (mg AA/kg fw)		PP ChAE (mg ChAc/kg fw)	
Sanitizing Treatment	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**
Control	405,8^{b,c,d}	29,3	0,0^{d}	0,0	1599,5^{a,b}	144,6
NaClO	456,5^{b}	34,6	0,0^{d}	0,0	1654,9^{a}	23,9
NEW	531,2^{a}	20,4	0,0^{d}	0,0	1387,2^{c,d}	113,9
UV-C	395,7^{c,d}	35,4	9,4^{c,d}	3,0	1584,3^{a,b}	117,8
NEW+UV-C	402,2^{b,c,d}	27,8	8,4^{c,d}	3,7	1526,4^{a,b,c}	55,4
HO_2	326,2^{e}	21,3	35,9^{b}	8,9	1375,9^{d}	68,7
UV-C+HO_2	353,1^{d,e}	25,5	43,1^{b}	8,3	1074,6^{e}	60,2
NEW+HO_2	430,4^{b,c}	80,2	18,1^{c}	6,8	1451,2^{b,c,d}	129,0
MIX	388,9^{c,d}	24,1	76,0^{a}	14,8	1547,3^{a,b}	108,4
	Palmitic Acid (µmol/g fw)		**Stearic Acid (µmol/g fw)**		**Linolenic Acid (µmol/g fw)**	
Sanitizing Treatment	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**
Control	1,4^{e}	0,2	0,2^{c}	0,0	3,0^{d}	0,2

NaClO	$2,2^{b,c}$	0,2	$0,4^{a,b}$	0,0	$4,0^{b,c}$	0,2
NEW	$2,0^{b,c}$	0,3	$0,3^{b}$	0,0	$3,8^{b,c}$	0,3
UV-C	$2,4^{a,b}$	0,2	$0,4^{a,b}$	0,1	$4,2^{b}$	0,1
NEW+UV-C	$1,8^{c,d}$	0,0	$0,2^{c}$	0,0	$3,5^{c,d}$	0,2
HO_2	$2,7^{a}$	0,1	$0,5^{a}$	0,0	$5,8^{a}$	0,3
UV-C+HO_2	$2,5^{a,b}$	0,1	$0,4^{b}$	0,0	$5,4^{a}$	0,4
NEW+HO_2	$2,8^{a}$	0,2	$0,5^{a}$	0,1	$5,4^{a}$	0,1
MIX	$1,7^{c,d}$	0,2	$0,3^{b}$	0,0	$3,8^{b,c}$	0,1

*a,b,c,d,e - homogeneous groups according to the non-parametric Mann-Whitney test for independent samples at 95% confidence level.

4 CONCLUSIONS

4.1 Total polyphenol content

The higher antioxidant activity in UV-C treatment on 9th day could be reported as an increase in its levels in a response of radiation exposure and PAL activity (Alothman, Bhat, & Karim, 2009).

Regarding neutral electrolyzed water a decrease was found in Bimi® but not it others cultivars of broccoli (Vandekinderen, et al., 2009). The same behavior was found in combined technique UV-C with superatmospheric oxygen MAP (Tomás-Callejas, Otón, Robles, Artés, & Artés-Hernández, 2012). Instead, to our knowledge there is no data linking those sanitizing methods on total polyphenol content in this new hybrid. For that reason, further researches should be taken to confirm the influence of modified atmposphere packing and electrolyzed water on phenolic constituents in *B. oleracea* (Italica × Alboglabra).

As main conclusion, all techniques had similar levels of total polyphenols, even though UV-C registered stable or slightly increasing values during 19 days of storage, which could be mentioned the best treatment for keeping total polyphenol content at 5°C, thus without significant differences ($p>0.05$) regarding the washing with chlorine and tap water.

4.2 Antioxidant Capacity Changes

4.2.1 Ferric Reducing Ability of Plasma (FRAP) Assay

The higher antioxidant activity in UV-C treatment on 9th day could be reported as a increase in its levels in a response of radiation exposure (Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011); however, more studies should be carried to verify its decrease after 19 days of storage, as well its effects as a combined treatment with Neutral Electrolyzed Water (NEW+UV-C). Notwithstanding, neutral electrolyzed water (NEW) has been mentioned with a slightly reduction on antioxidant levels in leafy vegetables (Aguayo, Boluda, Le Lann, & Artés, 2008) might be for its oxidative properties. The hereby observed low oxidation produced by NEW in Bimi® broccoli might be attributed to a lower penetration of the electrolyzed water in the inner cells of the tissue, contrary to the afore mentioned leafy vegetables.

As main conclusion, all treatments had similar activities, although the NEW treatment demonstrated stable or lightly increasing values after 9 days of storage time, and could be remarked the best treatment for keeping the antioxidant capacity at 5ºC with 95% of confidence level. Meanwhile the NEW+UV-C and UV-C treatment showed the lowest antioxidant activity (395.7-405.8 mg AAE kg^{-1} fw) without significant differences among them ($p>0.05$).

4.2.2 DPPH free-Radical Scavenging Activity Assay

Regarding samples in superatmospheric oxygen MAP, both single treatment and combined with Neutral Electrolyzed Water (NEW) showed similar trend on the matter of keeping the antioxidant power. Nevertheless, should be mentioned that to our knowledge no study have correlated modified atmosphere and antioxidant retention in Bimi® broccoli.

As principal conclusion, although all techniques had similar activities, the combined treatment MIX showed the least decreasing values during 19 of storage at 5°C, and could be highlighted the best treatment for maintaining antioxidant capacity and also with 95% of confidence level.

4.3 Fatty Acid Content

As a main conclusion, linolenic acid was the main fatty acid quantified in Bimi®, with a stable or slightly increasing trend during refrigerated shelf-life, after different sanitizing treatments. Superatmospheric O_2 presented a higher amount throughout shelf-life (2.68 µmol g^{-1} fw), being marked as the best treatment to preserve the set of fatty acids under evaluation.

5 LIST OF PUBLICATIONS AND COMMUNICATIONS

5.1 Publications

5.1.1 Article in peer-reviewed Scientific Journal

Martínez-Hernández, G.B., Artés-Hernández F., Gómez P.A., Formica A.C., Artés F. 2013. Synergistic combination of electrolyzed water, UV-C and superatmospheric O2 packaging for improving fresh-cut broccoli quality. *Postharvest Biology & Technology*. *76,* pp 125-134.

5.1.2 Full papers in peer-reviewed Proceedings

Formica, A. C., Martínez-Hernández, G. B., Artés-Hernández, F., Gómez, P., Cunha, L. M., & Artés, F. (2012). Total antioxidant capacity changes during shelf life in fresh-cut Bimi® broccoli alternatively sanitized. *VI Congreso Ibérico y IV Congreso Iberoamericano de Ciencias y Técnicas del Frío* (pp. 1-4). Madrid, Spain: Sociedad Española de Ciencias y Técnicas del Frío.

Formica-Oliveira, A. C., Martínez-Hernández, G B., Gómez, P., Artés-Hernández, F., Cunha, L. M., & Artés, F. (2012). Cambios en el contenido total de polifenoles del brócoli Bimi® mínimamente procesado bajo técnicas sostenibles de desinfección durante la vida útil. *X Simposio Nacional y VII Ibérico sobre Maduración y Postcosecha de Frutas y Hortalizas*, (p. in press). LLeida, Spain.

5.1.3 Abstracts

Formica, A. C., Martínez-Hernández, G. B., Artés-Hernández, F., Gómez, P., Cunha, L. M., & Artés, F. (2012). Total antioxidant capacity changes during shelf life in fresh-cut Bimi®

broccoli alternatively sanitized. *VI Congreso Ibérico y IV Congreso Iberoamericano de Ciencias y Técnicas del Frío, Avances en Ciencias y Tecnologías del Frío vol. IV (Book of Absrtracts),* (p. 106). Madrid, Spain: Sociedad Española de Ciencias y Técnicas del Frío.

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Gómez, P., Artés, F., Cunha, L. M., & Artés-Hernández, F. (2012). Antioxidant activity in minimally processed Bimi® Broccoli under different sanitizing techniques throughout shelf-life. *IJUP-2012, Book of Abstracts*, (p. 94). Porto: Universidade do Porto.

Martínez-Hernández, G., Formica, A., Gómez, P., Navarro-Rico, J., Falagán, N., Artés, F., et al. (2012). Extending the shelf life of the new Bimi® broccoli. *7th International Postharvest Symposium 2012 (IPS 2012), Book of Abstracts,* (p. 64). Kuala Lumpur, Malasya: Malaysian Agricultural Research and Development Institute.

Formica-Oliveira, A. C., Martínez-Hernández, G B., Gómez, P., Artés-Hernández, F., Cunha, L. M., & Artés, F. (2012). Total polyphenols content changes after sustainable combined sanitizing methods in minimally processed Bimi® broccoli during shelf life. *X Simposio Nacional y VII Ibérico sobre Maduración y Postcosecha de Frutas y Hortalizas*, (p. in press). LLeida, Spain.

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Artés, F., Gómez, P., Cunha, L. M., & Artés-Hernández, F. (2012). Characterization of fatty acid content in fresh-cut Bimi® broccoli sanitized under alternative techniques. *EFFoST 2012*, (p. in press). Montpellier, France.

5.2 Communications

5.2.1 Oral Presentations

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Gómez, P., Artés, F., Cunha, L. M., & Artés-Hernández, F. (2012). Antioxidant activity in minimally processed Bimi® Broccoli under different sanitizing techniques throughout shelf-life. *IJUP-2012*. Porto: Universidade do Porto.

Formica-Oliveira, A. C., Martínez-Hernández, G B., Gómez, P., Artés-Hernández, F., Cunha, L. M., & Artés, F. (2012). Total polyphenols content changes after sustainable combined

sanitizing methods in minimally processed Bimi® broccoli during shelf life. *X Simposio Nacional y VII Ibérico sobre Maduración y Postcosecha de Frutas y Hortalizas*. LLeida, Spain.

5.2.2 Poster Presentations

Formica, A. C., Martínez-Hernández, G. B., Artés-Hernández, F., Gómez, P., Cunha, L. M., & Artés, F. (2012). Cambios en el contenido total de polifenoles del brócoli Bimi® mínimamente procesado bajo técnicas sostenibles de desinfección durante la vida útil. *VI Congreso Ibérico y IV Congreso Iberoamericano de Ciencias y Técnicas del Frío.* Madrid, Spain.

Martínez-Hernández, G., Formica, A., Gómez, P., Navarro-Rico, J., Falagán, N., Artés, F., et al. (2012). Extending the shelf life of the new Bimi® broccoli. *7th International Postharvest Symposium 2012 (IPS 2012)*. Kuala Lumpur, Malasya.

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Artés, F., Gómez, P., Cunha, L. M., & Artés-Hernández, F. (2012). Characterization of fatty acid content in fresh-cut Bimi® broccoli sanitized under alternative techniques. *EFFoST 2012*. Montpellier, France.

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APPENDICES

1. List of Abstracts

Total antioxidant capacity changes during shelf life in fresh-cut Bimi® broccoli alternatively sanitized

Anna Carolina Formica[1,2]; Ginés Benito Martínez-Hernández[1,3]; Francisco Artés-Hernández[1,3]; Perla Gómez[3]; Luis Miguel Cunha[2]; Francisco Artés[1,3]

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Key words: Minimally processed; FRAP; UV-C radiation; electrolyzed water; chlorine

Abstract

The actual worldwide drive for a healthier lifestyle has led to a rising demand for convenient fresh foods, free from additives, with high nutritional value, including antioxidant and free-radical scavenging properties, to be consumed both in food services and at home. Broccoli is one of the most popular and highly perishable vegetables, and an important contributor of antioxidants in the diet as vitamin C and polyphenols. Chlorine is the most widely sanitizer used on whole and fresh-cut vegetables, although it has some disadvantages that lead to research on new emerging alternatives. This study compares the total antioxidant capacity changes, during the shelf life, in Bimi® broccoli (*Brassica oleracea* Italica x Alboglabra) after several sanitizing techniques. Four treatments were used (Chlorine - NaOCl; Neutral Electrolyzed Water- NEW; Ultraviolet radiation- UV-C; and a mix of electrolyzed water and radiation- NEW+UV-C). A control washed with tap water was used. After fresh-cut processing, Bimi® was stored at 5°C in darkness and total antioxidant capacity (FRAP assay) was analysed on the processing day and after 5, 9, 15 and 19 days at 5ºC. Sanitizing treatments reduced the initial total antioxidant capacity of 722.3 mg ascorbic acid equivalent units (AAE) kg^{-1} fw recording NEW+UV-C and UV-C the lowest values with approximately 309 mg AAE kg^{-1} fw on the processing day. NaOCl responded with the maximum total antioxidant capacity after 9 days of storage (640.1 mg AAE kg^{-1} fw). However the NEW treatment registered the greatest total antioxidant content (531.2 mg AAE kg^{-1} fw) after 19 days. All treatments decreased the total amount of antioxidant capacity after 5 days, but subsequently, an increased was found after 9 days at 5ºC. On the processing day, Control showed 722.3 mg AAE kg^{-1} fw, nonetheless presented the higher decrease after 15 days (257.1 mg AAE kg^{-1} fw). On the other hand, NEW+UV-C showed constant antioxidant capacity behaviour during the refrigerated shelf life. As main conclusion, all treatments had similar activities, although the NEW treatment demonstrated stable or lightly increasing values after 9 days of shelf life, and could be remarked the best treatment for keeping the

antioxidant capacity at 5ºC. Meanwhile NEW+UV-C, UV-C and control treatments showed the lowest antioxidant activity (395.7-405.8 mg AAE kg^{-1} fw) without significant differences among them.

Antioxidant activity in minimally processed Bimi® Broccoli under different sanitizing techniques throughout shelf-life

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In the present days, consumers are faced with a diverse variety of minimally processed fruits and vegetables, where sensorial and nutritional quality plays a major role on marketing those products [1]. Broccoli is one of the most popular and highly perishable vegetables, and an important contributor of antioxidants in the diet. Although chlorine is the most widely sanitizer used in fresh-cut vegetables, it has some disadvantages that lead to research on alternative treatments [2]. This study shows the total antioxidant capacity changes during the shelf life in Bimi® broccoli (*Brassica oleracea* Italica x Alboglabra) after alternative sanitizing techniques. Four treatments were assayed (high oxygen active modified atmosphere packaging (MAP) – HO; a combination of neutral electrolyzed water and high oxygen MAP - NEW+HO; UV-C radiation plus high oxygen MAP- UV+HO; and the triple combination - NEW+UV-C+HO). As control, 100 ppm NaClO was used as sanitizer in the washing step. After fresh-cut processing, Bimi® was stored at 5°C in darkness and total antioxidant capacity under modified FRAP assay [3], was analysed on processing day and after 5, 9, 15 and 19 days at 5ºC. Sanitizing treatments reduced the initial total antioxidant capacity of 479.6 mg ascorbic acid equivalent units (AAE) kg^{-1} fw recording NEW+HO the lowest values with 307.8 mg AAE kg^{-1} fw on 5th day. UV+HO responded with the maximum total antioxidant capacity after 5 days of storage (737.8 mg AAE kg^{-1} fw), nonetheless presented the higher decrease after 19 days (353.1 mg AAE kg^{-1} fw). On the other hand, HO registered initial antioxidant activity of 495.4 mg AAE kg^{-1} fw, yet showed the lowest reduction after 19 days (326.2 mg AAE kg^{-1} fw). Control samples registered the greatest total antioxidant content (456.5 mg AAE kg^{-1} fw) after 19 days. All treatments reduced the total amount of antioxidant capacity after 9 days, but subsequently, an increased was found after 15 days at 5ºC. As main conclusion, although all treatments had a similar trend, the combination of NEW+UV-C+HO could be remarked the best treatment for keeping the total antioxidant capacity at 5°C.

Total polyphenols content changes after sustainable combined sanitizing methods in minimally processed Bimi® broccoli during shelf life

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Diet and nutrition are important factors in the promotion and maintenance of good health throughout the entire life. Knowledge of the nature of fresh-cut fruits and vegetables, as they relate to pre- and postharvest handling, processing, packaging, and storage, is essential for ensuring their safety, nutritional value, and maintaining quality. The reaction of chlorine with organic residues can result in the formation of carcinogenic byproducts. Therefore, several alternatives to chlorine have been examined in order to implant them in the fresh-cut industry. Vegetables of the *Brassicaceae* family are rich in polyphenols, flavanoids and glucosinolates, among other nutrients. The aim of this work was to study the total polyphenol content changes after several sanitizing techniques in fresh-cut Bimi® broccoli (*Brassica oleracea* Italica x Alboglabra) during its shelf life. Three treatments were used (Neutral Electrolyzed Water- NEW; Ultraviolet radiation- UV-C; and the mix NEW+UV-C). As control, washing with 100 ppm NaClO was used. After fresh-cut processing, Bimi® was stored at 5°C in darkness and total polyphenols content was analyzed on the processing day and after 5, 9, 15 and 19 days at 5ºC. Sanitizing treatments reduced the initial total polyphenols amount of 1,392 mg ChAE kg^{-1} fw in approximately 13-14% on the processing day, without differences among treatments. Generally throughout storage, an initial total polyphenols capacity decrease was observed until day 15. From day 15th until the end of storage period an increase was registered. As main conclusion, all treatments had similar activities, although UV-C treatment demonstrated stable or lightly increasing values after 19 days of shelf life, and could be remarked the best treatment for keeping the total polyphenols volume at 5ºC regarding the initial control values

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Extending the shelf life of the new Bimi® broccoli by controlled atmosphere storage

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Keywords: *Brassica oleracea* Italica Group x Alboglabra Group; stem firmness; colour; pH; stem benting; yellowing, atmosphere modification.

Abstract

The new Bimi® brocoli is a natural hybrid between kailan (*Brassica oleracea* Alboglabra Group) and coventional broccoli (*Brassica oleracea* Italica Group). This new *Brassica* has a tender stem (similar to asparagus) and a small floret. Its mild flavour (compared to conventional *cvs*) make this vegetable ideal for fresh-cut purposes, besides their nutritional benefits. However, Bimi® is very perishable, being yellowing, stem bent, off-odors and off-favours the main sensory quality parameters affected during its postharvest life. Controlled atmosphere storage (CA) has been described as a very effective technique to maintain broccoli quality. The aim of the present work was to study changes in the respiration rate, sensory quality, pH, titratable acidity, total soluble solids, stem firmness and colour changes under 5 different CO_2 controlled atmosphere storage (5, 10, 15, 20, 25 kPa CO_2 + with 10 kPa O_2 + balanced with N_2) throughout 27 days at 2, 5 and 8ºC. A control under air conditions was used. The initial respiration rates of 36, 43 and 50 mg CO_2 kg^{-1} h^{-1} decreased after minimal processing, reaching the lowest values after 6-8 days, and subsequently increasing from day 8 to the end of the shelf life with a rate of around 35% for all samples. The better sensory scores were reached under 10 kPa CO_2. However CA of $\geq$15 kPa CO_2 avoided stem bent during 27 days at 2 and 5ºC. Samples stored at 8ºC were excluded due to yellowing and the high stem bent observed. Low moisture loss (0.5-1%) was registered after 27 days for both storage temperatures. Stems showed a luminosity increase around 23% after 6 days, without differences at both temperatures. The initial pH (6.0) rose approximately 3.5% after 6 days at both temperatures, with no changes until the end of the storage. Initial titratable acidity (TA) values (0.18 g citric acid 100 mL^{-1}) did not show changes during 19 days at both storage temperatures. However, after 27 days TA content increased between 14-40% for both storage temperatures, showing the higher the CO_2 concentrations the lower the TA increases. No stem firmness and soluble solids content changes were found throughout storage at both temperatures. In conclussion, CA storage with 10-15 kPa CO_2 (+10 kPa O_2 balanced with N_2) provides great benefits for keeping the quality of Bimi® broccoli duruing cold storage, reaching an acceptable sensory quality after 27 days of shelf life at 2 to 5ºC.

Characterization of Fatty Acid Content in Fresh-Cut Bimi® Broccoli Sanitized under Alternative Techniques

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Abstract

Nowadays, with the busier lifestyle, consumer's interest towards healthy minimally processed fruits and vegetables is growing fast. Bimi® (B. oleracea Italica x Alboglabra) is a green vegetable similar to conventional broccoli with a long slender stem. Comparatively, also have high respiration and transpiration rates, and a short shelf-life. Nutritionally, Bimi® is high in nutraceutical compounds [1]. Chlorine is the most widely sanitizer used on fresh-cut vegetables, although it has some disadvantages that lead to research on new emerging alternatives [2,3]. The primary objective of this work was to quantify and compare the fatty acid (linolenic, estearic and palmitic acids) content in Bimi® under different sanitizing techniques. Four treatments were used (chlorine; Neutral Electrolyzed Water; UV- C radiation; packaging under superatmospheric oxygen). Control was washed with tap water. After minimal processing, Bimi® was stored away from light and fatty acid content was analysed on the processing day and after 5, 9, 15 and 19 days of storage at 5ºC. Control samples showed 8.43µmol g^{-1} fw of linolenic acid on the processing day, but showed the highest decrease on its content during shelf-life, mainly caused by lipid peroxidation. With UV-C, the initial content was 5.50 µmol g^{-1} fw. Nevertheless, presented the lowest decrease over storage, as well as with superatmospheric O2, although without significant differences ($p>0.05$). Regarding stearic acid content, all treatments responded with stable or slightly increasing values. While for palmitic acid content, NEW treatment responded with initial content of 2.23 µmol g^{-1} fw, conversely, presented a decrease of 10% on its content throughout storage, with all other treatments, remaining constant. As a main conclusion, linolenic acid was the main fatty acid quantified in Bimi®, with a stable or slightly increasing trend during refrigerated shelf-life, after different sanitizing treatments. Superatmospheric O2 presented a higher amount throughout shelf-life (2.68 µmol g^{-1} fw), being marked as the best treatment to preserve the set of fatty acids under evaluation.

References:

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2. Overall data

2.1. Fatty Acid Content

µmol /g fw	Treat.	Rep.	Weight (g)	Palmitic Ac.	Stearic Ac.	Linolenic Ac.
DAY 0	Control	A	0,21	3,661	0,53	8,6
		B	0,20	3,068	0,48	8,2
		C	0,21	3,500	0,50	8,5
	NaOCl	A	0,26	3,200	0,45	7,1
		B	0,21	3,100	0,49	7,59849124
		C	0,22	2,900	0,55	7
	NEW	A	0,20	2,400	0,48	7,1
		B	0,20	2,500	0,50	6,8
		C	0,20	1,800	0,51	6,4
	UV-C	A	0,26	3,200	0,55	7,1
		B	0,21	3,100	0,55	7,59849124
		C	0,22	2,900	0,60	7
	NEW+UV-C	A	0,22	2,569	0,45	5,58368308
		B	0,20	2,450	0,49	5,3
		C	0,22	2,900	0,55	4,8
DAY 5	Control	A	0,21	1,331	0,25	2,85738504
		B	0,20	1,693	0,31	3,58565608
		C	0,22	1,500	0,21	3,2
	NaOCl	A	0,20	2,000	0,50	5,1
		B	0,21	2,120	0,44	6,02
		C	0,21	1,800	0,46	5,24
	NEW	A	0,21	1,900	0,35	3,5
		B	0,20	1,850	0,29	3,12
		C	0,20	1,800	0,40	2,9
	HO2	A	0,20	3,063	0,46	6,57720567
		B	0,20	2,680	0,41	6,2
		C	0,20	2,800	0,47	6,1
	UV-C	A	0,20	2,250	0,30	3,5
		B	0,22	2,400	0,35	3,6
		C	0,21	1,600	0,26	3,9
	NEW+UV-C	A	0,20	2,921	0,53	5,5
		B	0,20	2,061	0,55	5,3
		C		2,500	0,51	5,5
	NEW+HO2	A	0,20	2,500	0,45	6,1
		B	0,20	2,120	0,55	5,8
		C	0,20	2,761	0,49	6,15048257
	UV-C+HO2	A	0,21	2,800	0,38	5,6
		B	0,20	3,217	0,40	5,3
		C	0,21	2,500	0,42	5,1
	MIX	A	0,21	2,500	0,40	4,7
		B	0,20	2,400	0,50	4,8

		C	0,20	2,400	0,40	5,1
DAY 9	Control	A	0,20	1,104	0,24	2,13280763
		B	0,20	0,691	0,25	2,4
		C	0,20	1,387	0,27	2,63151738
	NaOCl	A	0,21	2,697	0,49	4,4
		B	0,20	1,876	0,46	4,8
		C	0,21	2,304	0,50	4,23191554
	NEW	A	0,20	1,450	0,29	2,56656315
		B	0,20	1,400	0,34	2,4
		C	0,20	1,500	0,31	2,2
	HO2	A	0,20	2,630	0,51	5,3
		B	0,21	2,255	0,44	5
		C	0,20	2,286	0,46	5,1
	UV-C	A	0,21	2,500	0,25	2,5
		B	0,21	2,200	0,28	3,5
		C	0,21	1,900	0,24	3
	NEW+UV-C	A	0,20	2,700	0,47	5,4
		B	0,20	2,500	0,50	5,3
		C	0,20	2,200	0,45	5,1
	NEW+HO2	A	0,20	2,500	0,36	5,2
		B	0,21	2,800	0,40	4,8
		C	0,20	2,120	0,35	4,5
	UV-C+HO2	A	0,21	2,200	0,35	4,2
		B	0,20	1,900	0,35	3,8
		C	0,20	2,100	0,42	4,5
	MIX	A	0,20	0,878	0,25	2,8
		B	0,19	1,585	0,25	3,4
		C	0,21	1,779	0,28	3,2
DAY 15	Control	A	0,23	1,500	0,25	3,2
		B	0,21	1,571	0,22	3,5
		C	0,20	1,251	0,20	3,2
	NaOCl	A	0,21	1,522	0,25	3,1
		B	0,21	2,108	0,31	3,4
		C	0,20	2,078	0,29	3,55150556
	NEW	A	0,18	2,285	0,24	3,9
		B	0,16	2,000	0,26	3,6
		C	0,20	1,869	0,28	3,94987267
	HO2	A	0,21	2,410	0,45	5,89586395
		B	0,19	2,400	0,44	5,5
		C	0,21	2,350	0,46	6
	UV-C	A	0,24	1,608	0,25	3,5
		B	0,21	2,171	0,27	4,1
		C	0,22	2,009	0,31	4,20594191
	NEW+UV-C	A	0,21	2,200	0,38	4
		B	0,22	1,900	0,32	4,312687
		C	0,18	2,100	0,35	4,3
	NEW+HO2	A	0,18	2,267	0,37	4,60211606
		B	0,19	2,459	0,36	4,99256905
		C	0,22	2,430	0,41	4,81102163

	UV-C+HO2	A	0,20	2,171	0,30	4,8
		B	0,20	2,769	0,41	5,1
		C	0,20	2,613	0,38	5,41123765
	MIX	A	0,30	1,567	0,28	3,48737461
		B	0,18	1,459	0,20	3,22471569
		C	0,22	1,550	0,20	3,50197461
DAY 19	Control	A	0,21	1,200	0,19	3,2
		B	0,20	1,591	0,22	3,12004414
		C	0,21	1,400	0,20	2,8
	NaOCl	A	0,20	2,400	0,35	4,1
		B	0,22	2,000	0,36	4,2
		C	0,21	2,100	0,39	3,74262956
	NEW	A	0,20	1,900	0,36	4
		B	0,19	1,800	0,32	3,5
		C	0,21	2,328	0,35	3,8
	HO2	A	0,23	2,750	0,49	5,5
		B	0,21	2,600	0,45	5,8
		C	0,21	2,700	0,48	6,0996279
	UV-C	A	0,20	2,600	0,46	4,1
		B	0,20	2,402	0,35	4,2
		C		2,300	0,40	4,3
	NEW+UV-C	A	0,21	1,768	0,20	3,46117052
		B	0,21	1,799	0,17	3,44304588
		C	0,20	1,865	0,21	3,71149186
	NEW+HO2	A	0,22	2,900	0,45	5,3
		B	0,22	2,600	0,55	5,5
		C	0,20	2,800	0,41	5,4
	UV-C+HO2	A	0,21	2,500	0,40	5,8
		B	0,20	2,396	0,31	5,06746962
		C	0,23	2,600	0,35	5,4
	MIX	A	0,20	1,900	0,29	3,74
		B	0,21	1,800	0,34	3,9
		C	0,22	1,500	0,32	3,8

MEAN									
Palmitic acid									
Day	**Control**	**NaClO**	**NEW**	**HO2**	**UV-C**	**NEW+UV-C**	**NEW+HO2**	**UV-C+HO2**	**MIX**
0	3,410	3,067	2,233		2,707	2,640			
5	1,508	1,973	1,850	2,848	2,083	2,494	2,460	2,839	2,433
9	1,061	2,292	1,450	2,390	2,200	2,467	2,473	2,067	1,414
15	1,441	1,902	2,052	2,387	1,929	2,067	2,385	2,518	1,525
19	1,397	2,167	2,009	2,683	2,434	1,810	2,767	2,499	1,733
Stearic acid									
Day	**Control**	**NaClO**	**NEW**	**HO2**	**UV-C**	**NEW+UV-C**	**NEW+HO2**	**UV-C+HO2**	**MIX**
0	0,503	0,495	0,497		0,400	0,495			
5	0,257	0,467	0,347	0,447	0,303	0,530	0,498	0,400	0,433
9	0,254	0,484	0,313	0,472	0,255	0,473	0,370	0,373	0,260

15	0,222	0,283	0,259	0,448	0,275	0,351	0,380	0,365	0,227
19	0,203	0,365	0,343	0,472	0,401	0,193	0,470	0,354	0,317

Linolenic acid									
Day	**Control**	**NaClO**	**NEW**	**HO2**	**UV-C**	**NEW+UV-C**	**NEW+HO2**	**UV-C+HO2**	**MIX**
0	8,433	7,233	6,7667		5,5000	5,23			
5	3,214	5,453	3,1733	6,2924	3,6667	5,43	6,02	5,33	4,87
9	2,388	4,477	2,3889	5,1333	3,0000	5,27	4,83	4,17	3,13
15	3,300	3,351	3,8166	5,7986	3,9353	4,20	4,80	5,10	3,40
19	3,040	4,014	3,7667	5,7999	4,200	3,54	5,40	5,42	3,81

SD

Palmitic acid									
Day	**Control**	**NaOCl**	**NEW**	**HO2**	**UV-C**	**NEW+UV-C**	**NEW+HO2**	**UV-C+HO2**	**MIX**
0	0,306	0,153	0,379	0,153	0,153	0,233			
5	0,181	0,162	0,050	0,196	0,425	0,430	0,322	0,360	0,058
9	0,350	0,410	0,050	0,208	0,300	0,252	0,341	0,153	0,474
15	0,168	0,330	0,213	0,032	0,290	0,153	0,104	0,310	0,058
19	0,196	0,208	0,280	0,076	0,152	0,049	0,153	0,102	0,208

Stearic acid									
Day	**Control**	**NaOCl**	**NEW**	**HO2**	**UV-C**	**NEW+UV-C**	**NEW+HO2**	**UV-C+HO2**	**MIX**
0	0,03	0,05	0,02		0,03	0,05			
5	0,05	0,03	0,06	0,03	0,05	0,02	0,05	0,02	0,06
9	0,02	0,02	0,03	0,04	0,02	0,03	0,03	0,04	0,02
15	0,03	0,03	0,02	0,01	0,03	0,03	0,03	0,05	0,05
19	0,02	0,02	0,02	0,02	0,05	0,02	0,07	0,04	0,03

Linolenic acid									
Day	**Control**	**NaOCl**	**NEW**	**HO2**	**UV-C**	**NEW+UV-C**	**NEW+HO2**	**UV-C+HO2**	**MIX**
0	0,208	0,321	0,351	0,321	0,321	0,397			
5	0,364	0,496	0,304	0,252	0,208	0,115	0,189	0,252	0,208
9	0,250	0,292	0,184	0,153	0,500	0,153	0,351	0,351	0,306
15	0,173	0,230	0,189	0,264	0,381	0,177	0,195	0,306	0,156
19	0,212	0,240	0,252	0,300	0,100	0,150	0,100	0,367	0,081

2.2. Total Polyphenol Content

Day 0									
		Rep (plat)	**Weight (g)**	**Absorb.**	**Abs - Blank**	**mg Clor/ml sample**	**mg ChAE/ kg fw**	**Mean**	**St. Dev**
Control	A	1	0,5120	0,103	0,056	0,021	1458,395	**1391,6**	
		2	0,5120	0,102	0,056	0,021	1439,298		**99,7**

		3	0,5120	0,097	0,050	0,019	1276,978		
	B	1	0,5027	0,104	0,057	0,022	1510,539		
		2	0,5027	0,102	0,055	0,021	1449,111	**1555,8**	
		3	0,5027	0,110	0,063	0,025	1707,755		**135,1**
	C	1	0,5040	0,113	0,067	0,026	1823,341		
		2	0,5040	0,110	0,063	0,025	1703,982	**1753,4**	
		3	0,5040	0,111	0,064	0,025	1733,015		**62,2**
	A	1	0,4940	0,429	0,382	0,173	1220,906		
		2	0,4940	0,424	0,378	0,171	1206,138	**1221,6**	
		3	0,4940	0,434	0,387	0,175	1237,644		**15,8**
	B	1	0,5354	0,498	0,451	0,205	1350,553		
NaOCl		2	0,5354	0,505	0,459	0,208	1374,237	**1380,9**	
		3	0,5354	0,520	0,473	0,215	1418,051		**34,2**
	C	1	0,47474	0,486	0,440	0,199	1458,373		
		2	0,47474	0,488	0,441	0,200	1463,807	**1469,5**	
		3	0,47474	0,494	0,448	0,203	1486,221		**14,8**
	A	1	0,4904	0,461	0,415	0,188	1336,263		
		2	0,4904	0,459	0,413	0,187	1329,328	**1332,1**	
		3	0,4904	0,460	0,413	0,187	1330,649		**3,7**
	B	1	0,5241	0,477	0,431	0,195	1311,396		
NEW		2	0,5241	0,484	0,438	0,198	1333,860	**1332,9**	
		3	0,5241	0,491	0,444	0,201	1353,516		**21,1**
	C	1	0,5429	0,462	0,416	0,188	1227,015		
		2	0,5429	0,458	0,411	0,186	1214,297	**1228,8**	
		3	0,5429	0,468	0,422	0,191	1245,183		**15,5**
	A	1	0,5249	0,499	0,452	0,205	1377,622		
		2	0,5249	0,499	0,453	0,205	1378,557	**1376,4**	
		3	0,5249	0,497	0,451	0,204	1372,948		**3,0**
	B	1	0,4893	0,420	0,374	0,169	1201,858		
UV-C		2	0,4893	0,429	0,383	0,173	1231,638	**1224,1**	
		3	0,4893	0,431	0,385	0,174	1238,917		**19,6**
	C	1	0,48418	0,450	0,403	0,182	1312,286		
		2	0,48418	0,437	0,391	0,176	1269,580	**1284,9**	
		3	0,48418	0,438	0,392	0,177	1272,886		**23,8**
NEW+UV-C	A	1	0,5041	0,468	0,421	0,191	1325,047	**1293,8**	
		2	0,5041	0,452	0,406	0,184	1276,344		**27,2**

		3	0,5041	0,454	0,407	0,184	1279,892		
	B	1	0,5391	0,475	0,429	0,194	1274,242		
		2	0,5391	0,485	0,439	0,199	1304,399	**1279,8**	
		3	0,5391	0,471	0,424	0,192	1260,840		**22,3**
	C	1	0,4906	0,489	0,443	0,201	1426,912		
		2	0,4906	0,481	0,435	0,197	1401,822	**1398,3**	
		3	0,4906	0,471	0,424	0,192	1366,167		**30,5**

Day 5									
		Rep (plat)	**Weight(g)**	**Abs.**	**Abs - Blank**	**mg Clor/ml samp**	**mg ChAE/ kg fw**	**Mean**	**St. Dev.**
	A	1	0,5146	0,088	0,041	0,014	973,579		
		2	0,5146	0,088	0,041	0,014	979,917	**954,6**	
		3	0,5146	0,086	0,039	0,013	910,199		**38,6**
	B	1	0,5090	0,107	0,060	0,023	1603,284		
Control		2	0,5090	0,108	0,062	0,024	1651,266	**1744,0**	
		3	0,5090	0,119	0,072	0,029	1977,541		**203,6**
	C	1	0,5060	0,106	0,060	0,023	1598,551		
		2	0,5060	0,105	0,058	0,022	1537,466	**1623,2**	
		3	0,5060	0,111	0,064	0,025	1733,581		**100,4**
	A	1	0,5081	0,101	0,055	0,021	1419,902		
		2	0,5081	0,100	0,053	0,020	1371,848	**1464,8**	
		3	0,5081	0,107	0,060	0,023	1602,509		**121,7**
	B	1	0,5000	0,109	0,062	0,024	1686,421		
NaOCl		2	0,5000	0,116	0,070	0,028	1926,773	**1855,3**	**146,8**
		3	0,5000	0,117	0,070	0,028	1952,757		
	C	1	0,4992	0,107	0,060	0,023	1620,436	**1803,3**	**462,7**
		2	0,4992	0,102	0,055	0,021	1460,090		
		3	0,4992	0,128	0,082	0,033	2329,473		
	A	1	0,49495	0,086	0,040	0,014	957,426		
		2	0,49495	0,087	0,040	0,014	986,914	**995,7**	
		3	0,49495	0,089	0,042	0,015	1042,612		**43,3**
	B	1	0,5105	0,110	0,064	0,025	1710,933		
HO2		2	0,5105	0,110	0,064	0,025	1704,551	**1712,0**	
		3	0,5105	0,111	0,064	0,025	1720,505		**8,0**
	C	1	0,4990	0,106	0,059	0,023	1589,759		
		2	0,4990	0,116	0,069	0,027	1922,177	**1710,8**	
		3	0,4990	0,107	0,060	0,023	1620,342		**183,7**

	A	1	0,5160	0,393	0,346	0,156	1061,759		
		2	0,5160	0,386	0,339	0,153	1040,197	**1055,0**	
		3	0,5160	0,393	0,347	0,156	1063,024		**12,8**
	B	1	0,4864	0,400	0,353	0,159	1141,169		
NEW		2	0,4864	0,394	0,348	0,157	1122,877	**1144,4**	
		3	0,4864	0,408	0,362	0,163	1169,106		**23,3**
	C	1	0,4841	0,515	0,468	0,212	1529,202		
		2	0,4841	0,512	0,466	0,211	1520,519	**1525,9**	
		3	0,4841	0,514	0,468	0,212	1528,047		**4,7**
	A	1	0,51263	0,393	0,346	0,156	1067,715		
		2	0,51263	0,390	0,344	0,155	1059,766	**1066,3**	
		3	0,51263	0,394	0,347	0,156	1071,530		**6,0**
	B	1	0,4840	0,561	0,515	0,234	1684,067	**1688,2**	**3,8**
UV-C		2	0,4840	0,563	0,517	0,235	1691,616		
		3	0,4840	0,562	0,516	0,235	1688,810		
	C	1	0,5037	0,549	0,502	0,228	1587,799	**1570,4**	**26,2**
		2	0,5037	0,547	0,501	0,228	1583,184		
		3	0,5037	0,534	0,488	0,221	1540,256		
	A	1	0,5269	0,475	0,428	0,194	1298,631		
		2	0,5269	0,482	0,435	0,197	1320,372	**1308,8**	
		3	0,5269	0,478	0,431	0,195	1307,327		**10,9**
	B	1	0,5050	0,431	0,385	0,174	1205,801		
NEW+HO2		2	0,5050	0,432	0,385	0,174	1207,283	**1207,0**	
		3	0,5050	0,432	0,385	0,174	1208,056		**1,1**
	C	1	0,4935	0,475	0,429	0,194	1375,330		
		2	0,4935	0,481	0,434	0,197	1392,771	**1380,8**	
		3	0,4935	0,475	0,429	0,194	1374,394		**10,4**
	A	1	0,4847	0,450	0,404	0,183	1313,409		
		2	0,4847	0,444	0,397	0,180	1291,025	**1341,1**	
		3	0,4847	0,482	0,436	0,197	1418,822		**68,2**
	B	1	0,5316	0,535	0,488	0,222	1473,510		
NEW+UV-C		2	0,5316	0,531	0,485	0,220	1462,718	**1469,7**	
		3	0,5316	0,535	0,488	0,222	1472,891		**6,1**
	C	1	0,4753	0,452	0,406	0,183	1340,690		
		2	0,4753	0,454	0,408	0,184	1347,577	**1360,0**	
		3	0,4753	0,467	0,421	0,190	1391,750		**27,7**
UV-C+HO2	A	1	0,4975	0,450	0,404	0,183	1283,664	**1295,5**	**14,5**

		2	0,4975	0,459	0,412	0,187	1311,717		
		3	0,4975	0,453	0,406	0,184	1291,167		
	B	1	0,4750	0,474	0,428	0,194	1416,949		
		2	0,4750	0,500	0,454	0,206	1505,513	**1461,2**	
		3	0,4750	0,509	0,463	0,210	1536,097		**62,6**
	C	1	0,4953	0,505	0,458	0,208	1466,127		
		2	0,4953	0,514	0,467	0,212	1496,415	**1471,9**	
		3	0,4953	0,501	0,454	0,206	1453,258		**22,2**
	A	1	0,5230	0,454	0,408	0,184	1242,794		
		2	0,5230	0,458	0,411	0,186	1253,733	**1243,1**	
		3	0,5230	0,451	0,405	0,183	1232,761		**10,5**
	B	1	0,5007	0,412	0,365	0,165	1152,054		
MIX		2	0,5007	0,414	0,367	0,166	1157,864	**1165,5**	
		3	0,5007	0,423	0,376	0,170	1186,441		**18,4**
	C	1	0,5037	0,473	0,426	0,193	1342,989		
		2	0,5037	0,464	0,417	0,189	1313,038	**1339,4**	
		3	0,5037	0,479	0,432	0,196	1362,096		**24,7**

Day 9									
		Rep. (plat.)	**Weight (g)**	**Abs.**	**Abs - Blanco**	**mg Clor/ml sample**	**mg ChAE/ kg fw**	**Mean**	**St. Dev.**
	A	1	0,4844	0,079	0,032	0,010	725,001		
		2	0,4844	0,079	0,032	0,010	735,014	**719,4**	
		3	0,4844	0,078	0,031	0,010	698,299		**19,0**
	B	1	0,5061	0,107	0,060	0,023	1611,139		
Control		2	0,5061	0,108	0,062	0,024	1659,355	**1752,6**	
		3	0,5061	0,119	0,072	0,029	1987,229		**204,6**
	C	1	0,4953	0,100	0,053	0,020	1405,440		
		2	0,4953	0,106	0,060	0,023	1611,729	**1460,0**	
		3	0,4953	0,098	0,052	0,019	1362,873		**133,1**
	A	1	0,5101	0,093	0,046	0,017	1153,326		
		2	0,5101	0,092	0,046	0,016	1130,976	**1143,7**	
		3	0,5101	0,093	0,046	0,017	1146,940		**11,5**
	B	1	0,5113	0,111	0,064	0,025	1711,831		
NaOCl		2	0,5113	0,115	0,069	0,027	1867,969	**1818,0**	
		3	0,5113	0,116	0,069	0,027	1874,341		**92,0**
	C	1	0,4976	0,104	0,057	0,022	1536,845		
		2	0,4976	0,106	0,060	0,023	1618,380	**1521,6**	
		3	0,4976	0,100	0,054	0,020	1409,649		**105,2**

	A	1	0,5119	0,077	0,031	0,010	653,268		
		2	0,5119	0,078	0,031	0,010	659,634	**666,0**	
		3	0,5119	0,078	0,032	0,010	685,101		**16,8**
	B	1	0,4862	0,108	0,061	0,024	1690,853		
HO2		2	0,4862	0,117	0,071	0,028	2003,592	**1762,9**	
		3	0,4862	0,105	0,058	0,022	1594,369		**213,9**
	C	1	0,5045	0,109	0,062	0,024	1670,305		
		2	0,5045	0,107	0,060	0,023	1612,288	**1657,4**	
		3	0,5045	0,109	0,063	0,024	1689,644		**40,3**
	A	1	0,4855	0,514	0,467	0,212	1521,741		
		2	0,4855	0,515	0,468	0,213	1526,038	**1523,6**	
		3	0,4855	0,514	0,468	0,212	1523,007		**2,2**
	B	1	0,4864	0,495	0,449	0,203	1457,457		
NEW		2	0,4864	0,495	0,448	0,203	1456,126	**1460,3**	
		3	0,4864	0,498	0,452	0,205	1467,434		**6,2**
	C	1	0,5097	0,472	0,426	0,193	1326,641		
		2	0,5097	0,470	0,424	0,192	1321,274	**1331,5**	
		3	0,5097	0,478	0,432	0,196	1346,450		**13,3**
	A	1	0,5034	0,468	0,422	0,191	1328,239		
		2	0,5034	0,467	0,421	0,190	1325,656	**1327,2**	
		3	0,5034	0,468	0,422	0,191	1327,819		**1,4**
	B	1	0,4977	0,362	0,315	0,142	994,961		
UV-C		2	0,4977	0,364	0,318	0,143	1001,995	**999,0**	
		3	0,4977	0,363	0,317	0,142	1000,179		**3,7**
	C	1	0,5051	0,480	0,433	0,196	1362,084		
		2	0,5051	0,475	0,429	0,194	1347,308	**1352,2**	
		3	0,5051	0,475	0,429	0,194	1347,214		**8,6**
	A	1	0,4945	0,492	0,446	0,202	1427,405		
		2	0,4945	0,497	0,451	0,204	1443,800	**1441,1**	
		3	0,4945	0,500	0,453	0,205	1451,997		**12,5**
	B	1	0,5050	0,437	0,391	0,177	1225,897		
NEW+HO2		2	0,5050	0,422	0,375	0,169	1175,851	**1190,6**	
		3	0,5050	0,420	0,374	0,169	1170,055		**30,7**
	C	1	0,5097	0,509	0,462	0,210	1443,739		
		2	0,5097	0,509	0,463	0,210	1444,857	**1442,4**	
		3	0,5097	0,507	0,461	0,209	1438,595		**3,3**

NEW+UV-C	A	1	0,4915	0,391	0,345	0,155	1101,645		
		2	0,4915	0,379	0,332	0,149	1060,442	**1086,9**	
		3	0,4915	0,390	0,344	0,155	1098,678		**23,0**
	B	1	0,5234	0,411	0,365	0,164	1107,361		
		2	0,5234	0,407	0,361	0,163	1094,305	**1104,5**	
		3	0,5234	0,413	0,366	0,165	1111,984		**9,2**
	C	1	0,4853	0,386	0,340	0,153	1097,076		
		2	0,4853	0,394	0,348	0,156	1123,734	**1123,4**	
		3	0,4853	0,402	0,355	0,160	1149,493		**26,2**
UV-C+HO2	A	1	0,4798	0,420	0,373	0,168	1221,644		
		2	0,4798	0,416	0,370	0,167	1210,270	**1215,2**	
		3	0,4798	0,417	0,371	0,167	1213,702		**5,8**
	B	1	0,5236	0,385	0,339	0,152	1024,879		
		2	0,5236	0,369	0,323	0,145	974,919	**998,8**	
		3	0,5236	0,376	0,329	0,148	996,464		**25,1**
	C	1	0,5299	0,409	0,363	0,164	1090,074		
		2	0,5299	0,395	0,349	0,157	1045,414	**1075,9**	
		3	0,5299	0,410	0,364	0,164	1092,086		**26,4**
MIX	A	1	0,5011	0,403	0,357	0,161	1123,502		
		2	0,5011	0,405	0,359	0,162	1130,193	**1129,5**	
		3	0,5011	0,407	0,360	0,162	1134,732		**5,6**
	B	1	0,5007	0,416	0,370	0,167	1165,354		
		2	0,5007	0,414	0,367	0,166	1157,990	**1165,3**	
		3	0,5007	0,418	0,372	0,168	1172,556		**7,3**
	C	1	0,5254	0,429	0,382	0,173	1158,568		
		2	0,5254	0,421	0,375	0,169	1134,159	**1138,2**	
		3	0,5254	0,417	0,371	0,167	1121,830		**18,7**

Day 15									
		Rep (plat)	**Weight (g)**	**Abs.**	**Abs - Blanco**	**mg Clor/ml sample**	**mg ChAE/kg fw**	**Mean**	**St. Dev**
Control	A	1	0,4910	0,520	0,474	0,215	1528,182		
		2	0,4910	0,502	0,456	0,207	1468,800	**1503,5**	
		3	0,4910	0,516	0,469	0,213	1513,667		**31,0**
	B	1	0,5231	0,511	0,465	0,211	1419,470		
		2	0,5231	0,491	0,445	0,201	1356,969	**1375,9**	
		3	0,5231	0,489	0,443	0,201	1351,344		**37,8**
	C	1	0,5224	0,509	0,463	0,210	1415,145	**1408,9**	
		2	0,5224	0,508	0,461	0,209	1410,452		**7,2**

		3	0,5224	0,505	0,458	0,208	1401,095		
NaOCl	A	1	0,5023	0,452	0,406	0,183	1279,289		
		2	0,5023	0,454	0,408	0,184	1285,178	**1280,6**	
		3	0,5023	0,452	0,405	0,183	1277,219		**4,1**
	B	1	0,4815	0,406	0,360	0,162	1172,634		
		2	0,4815	0,407	0,360	0,162	1174,311	**1168,3**	
		3	0,4815	0,402	0,355	0,160	1157,872		**9,0**
	C	1	0,4880	0,392	0,345	0,155	1111,086		
		2	0,4880	0,401	0,354	0,160	1140,934	**1140,6**	
		3	0,4880	0,410	0,363	0,164	1169,788		**29,4**
HO2	A	1	0,4731	0,430	0,384	0,173	1271,644		
		2	0,4731	0,446	0,399	0,180	1324,980	**1308,0**	
		3	0,4731	0,447	0,400	0,181	1327,508		**31,5**
	B	1	0,4929	0,419	0,373	0,168	1191,064		
		2	0,4929	0,411	0,365	0,164	1164,758	**1175,5**	
		3	0,4929	0,413	0,366	0,165	1170,677		**13,8**
	C	1	0,4908	0,438	0,391	0,177	1256,497		
		2	0,4908	0,431	0,384	0,174	1234,385	**1248,8**	
		3	0,4908	0,437	0,391	0,177	1255,573		**12,5**
NEW	A	1	0,4911	0,461	0,415	0,188	1333,637		
		2	0,4911	0,472	0,426	0,193	1369,590	**1347,5**	
		3	0,4911	0,463	0,416	0,188	1339,244		**19,3**
	B	1	0,4914	0,464	0,417	0,189	1342,076		
		2	0,4914	0,458	0,411	0,186	1322,058	**1334,6**	
		3	0,4914	0,463	0,417	0,189	1339,771		**11,0**
	C	1	0,5376	0,465	0,419	0,189	1246,418		
		2	0,5376	0,451	0,405	0,183	1204,771	**1231,6**	
		3	0,5376	0,464	0,418	0,189	1243,670		**23,3**
UV-C	A	1	0,4956	0,464	0,417	0,189	1332,283		
		2	0,4956	0,470	0,423	0,192	1351,867	**1333,8**	
		3	0,4956	0,459	0,413	0,187	1317,340		**17,3**
	B	1	0,5319	0,454	0,408	0,184	1224,316		
		2	0,5319	0,447	0,400	0,181	1201,054	**1223,9**	
		3	0,5319	0,461	0,415	0,188	1246,345		**22,6**
	C	1	0,5274	0,454	0,408	0,184	1232,879	**1244,6**	
		2	0,5274	0,451	0,404	0,183	1223,103		**29,1**

		3	0,5274	0,468	0,422	0,191	1277,722		
	A	1	0,5125	0,447	0,401	0,181	1241,262		
		2	0,5125	0,443	0,396	0,179	1227,978	**1228,7**	
		3	0,5125	0,439	0,393	0,178	1216,838		**12,2**
	B	1	0,5050	0,454	0,408	0,184	1280,516		
NEW+HO2		2	0,5050	0,448	0,402	0,182	1260,549	**1271,8**	
		3	0,5050	0,452	0,406	0,184	1274,397		**10,2**
	C	1	0,5272	0,507	0,461	0,209	1398,584		
		2	0,5272	0,500	0,454	0,206	1376,232	**1388,0**	
		3	0,5272	0,504	0,458	0,208	1389,156		**11,2**
	A	1	0,4964	0,430	0,384	0,173	1219,564		
		2	0,4964	0,430	0,384	0,173	1219,753	**1226,9**	
		3	0,4964	0,437	0,390	0,176	1241,330		**12,5**
	B	1	0,4925	0,401	0,354	0,160	1131,968		
NEW+UV-C		2	0,4925	0,392	0,345	0,155	1101,433	**1129,5**	
		3	0,4925	0,408	0,361	0,163	1155,001		**26,9**
	C	1	0,5070	0,389	0,342	0,154	1064,967		
		2	0,5070	0,395	0,349	0,157	1085,828	**1076,2**	
		3	0,5070	0,393	0,346	0,156	1077,805		**10,5**
	A	1	0,4833	0,469	0,422	0,191	1376,880		
		2	0,4833	0,470	0,423	0,192	1381,227	**1386,4**	
		3	0,4833	0,476	0,429	0,194	1400,958		**12,8**
	B	1	0,4821	0,451	0,405	0,183	1321,216		
UV-C+HO2		2	0,4821	0,468	0,422	0,191	1378,189	**1357,0**	
		3	0,4821	0,466	0,420	0,190	1371,453		**31,1**
	C	1	0,4508	0,458	0,412	0,186	1425,077		
		2	0,4508	0,458	0,412	0,186	1426,143	**1416,1**	
		3	0,4508	0,450	0,404	0,183	1397,018		**16,5**
	A	1	0,5040	0,498	0,452	0,205	1423,727		
		2	0,5040	0,503	0,456	0,207	1439,147	**1420,4**	
		3	0,5040	0,490	0,444	0,201	1398,178		**20,7**
MIX	B	1	0,5149	0,500	0,454	0,206	1403,847		
		2	0,5149	0,500	0,454	0,206	1403,875	**1407,4**	
		3	0,5149	0,503	0,457	0,207	1414,346		**6,1**
	C	1	0,4965	0,497	0,450	0,204	1437,173	**1445,8**	
		2	0,4965	0,497	0,450	0,204	1438,153		**14,1**

		3	0,4965	0,504	0,458	0,208	1462,007		

Day 19									
		Rep (plat)	**Weight (g)**	**Abs.**	**Abs - Blank**	**mg Clor/ ml sample**	**mg ChAE/ kg fw**	**Promédio**	**Desv Est**
	A	1	0,5013	0,498	0,451	0,205	1429,579		
		2	0,5013	0,491	0,445	0,201	1407,218	**1419,3**	
		3	0,5013	0,495	0,449	0,203	1420,991		**11,3**
	B	1	0,4682	0,568	0,521	0,237	1755,637		
Control		2	0,4682	0,565	0,518	0,236	1746,116	**1748,4**	
		3	0,4682	0,564	0,518	0,235	1743,366		**6,4**
	C	1	0,5049	0,563	0,517	0,235	1630,532	**1631,0**	**5,2**
		2	0,5049	0,562	0,515	0,234	1626,054		
		3	0,5049	0,565	0,518	0,236	1636,329		
	A	1	0,5074	0,577	0,531	0,242	1669,841		
		2	0,5074	0,572	0,525	0,239	1651,559	**1648,7**	
		3	0,5074	0,563	0,517	0,235	1624,649		**22,7**
	B	1	0,4809	0,560	0,513	0,233	1688,764		
NaOCl		2	0,4809	0,552	0,506	0,230	1664,683	**1671,2**	
		3	0,4809	0,551	0,505	0,229	1660,216		**15,4**
	C	1	0,5085	0,581	0,535	0,243	1679,878		
		2	0,5085	0,565	0,519	0,236	1627,054	**1644,9**	
		3	0,5085	0,565	0,519	0,236	1627,790		**30,3**
	A	1	0,5266	0,473	0,427	0,193	1293,481		
		2	0,5266	0,488	0,442	0,200	1341,521	**1301,4**	
		3	0,5266	0,465	0,419	0,190	1269,119		**36,8**
	B	1	0,5143	0,497	0,451	0,204	1396,939		
HO2		2	0,5143	0,497	0,450	0,204	1394,859	**1395,0**	
		3	0,5143	0,496	0,450	0,204	1393,255		**1,8**
	C	1	0,5010	0,476	0,429	0,194	1358,979		
		2	0,5010	0,513	0,467	0,212	1479,111	**1431,2**	
		3	0,5010	0,506	0,459	0,208	1455,604		**63,7**
	A	1	0,5149	0,526	0,480	0,218	1486,548		
NEW		2	0,5149	0,527	0,481	0,218	1489,715	**1461,6**	
		3	0,5149	0,502	0,455	0,206	1408,629		**45,9**
	B	1	0,4988	0,510	0,463	0,210	1474,124	**1460,3**	**12,5**

		2	0,4988	0,504	0,458	0,208	1456,711		
		3	0,4988	0,502	0,456	0,207	1449,974		
	C	1	0,4837	0,423	0,376	0,170	1222,597		
		2	0,4837	0,424	0,377	0,170	1225,939	**1239,8**	
		3	0,4837	0,437	0,391	0,176	1270,722		**26,9**
	A	1	0,4888	0,494	0,447	0,203	1446,894		
		2	0,4888	0,492	0,445	0,202	1440,039	**1442,1**	
		3	0,4888	0,491	0,445	0,202	1439,376		**4,2**
	B	1	0,4774	0,556	0,509	0,232	1686,929		
UV-C		2	0,4774	0,568	0,521	0,237	1727,419	**1711,0**	
		3	0,4774	0,565	0,519	0,236	1718,699		**21,3**
	C	1	0,5092	0,560	0,513	0,233	1608,833		
		2	0,5092	0,560	0,513	0,233	1608,833	**1599,9**	
		3	0,5092	0,551	0,505	0,230	1581,972		**15,5**
	A	1	0,4980	0,454	0,407	0,184	1293,314		
		2	0,4980	0,443	0,397	0,179	1258,767	**1285,5**	
		3	0,4980	0,457	0,411	0,186	1304,395		**23,8**
	B	1	0,5043	0,546	0,499	0,227	1576,541		
NEW+HO2		2	0,5043	0,537	0,490	0,223	1547,716	**1569,3**	
		3	0,5043	0,548	0,501	0,228	1583,505		**19,0**
	C	1	0,5069	0,526	0,480	0,218	1507,856		
		2	0,5069	0,520	0,473	0,215	1486,637	**1498,9**	
		3	0,5069	0,525	0,478	0,217	1502,078		**11,0**
	A	1	0,4829	0,519	0,472	0,214	1545,526	**1552,6**	**10,5**
		2	0,4829	0,524	0,478	0,217	1564,601		
		3	0,4829	0,519	0,473	0,215	1547,534		
	B	1	0,4572	0,504	0,458	0,208	1569,482	**1570,8**	**13,2**
NEW+UV-C		2	0,4572	0,508	0,462	0,210	1584,569		
		3	0,4572	0,501	0,454	0,206	1558,255		
	C	1	0,5029	0,503	0,456	0,207	1441,745		
		2	0,5029	0,516	0,469	0,213	1483,211	**1455,9**	
		3	0,5029	0,503	0,457	0,207	1442,811		**23,6**
	A	1	0,5128	0,370	0,323	0,145	994,625		
		2	0,5128	0,370	0,324	0,146	997,168	**1026,4**	
UV-C+HO2		3	0,5128	0,399	0,352	0,159	1087,437		**52,9**
	B	1	0,5140	0,384	0,337	0,152	1036,418	**1059,9**	
		2	0,5140	0,393	0,346	0,156	1065,602		**21,2**

		3	0,5140	0,396	0,350	0,158	1077,561		
	C	1	0,5087	0,405	0,358	0,161	1113,036		
		2	0,5087	0,406	0,360	0,162	1118,234	**1137,6**	
		3	0,5087	0,426	0,380	0,171	1181,653		**38,2**
	A	1	0,5209	0,563	0,517	0,235	1588,196		
		2	0,5209	0,562	0,516	0,235	1585,436	**1593,0**	
		3	0,5209	0,569	0,522	0,237	1605,226		**10,7**
	B	1	0,5208	0,503	0,456	0,207	1398,772		
MIX		2	0,5208	0,505	0,458	0,208	1405,517	**1406,4**	
		3	0,5208	0,508	0,461	0,209	1414,770		**8,0**
	C	1	0,5023	0,570	0,523	0,238	1659,755		
		2	0,5023	0,563	0,516	0,235	1637,432	**1642,7**	
		3	0,5023	0,561	0,514	0,234	1630,961		**15,1**

2.3. Antioxidant Capacity

2.3.1. Ferric-Reducing Ability of Plasma (FRAP) Assay

Day 0									
		Rep (plat)	**Weight (g)**	**Abs.**	**Abs - Blanco**	**ml AA/ml sample**	**mg AAE/ kg fw**	**Mean**	**St. Dev**
	A	1	0,5120	0,592500002	0,3682	0,113977696	781,8158		
		2	0,5120	0,586600006	0,3623	0,112515492	771,7860	**781,5891684**	
		3	0,5120	0,598000121	0,3737	0,115340799	791,1658		**9,691908416**
	B	1	0,5027	0,48269999	0,2584	0,086765798	604,5645		
Control		2	0,5027	0,496499994	0,2722	0,090185873	628,3948	**622,926484**	
		3	0,5027	0,500800014	0,2765	0,091251553	635,8202		**16,32963188**
	C	1	0,5040	0,570100009	0,3458	0,108426273	753,8208		
		2	0,5040	0,586000001	0,3617	0,112366792	781,2167	**762,3783997**	
		3	0,5040	0,569099988	0,3448	0,108178436	752,0977		**16,33721471**
	A	1	0,4940	0,270799994	0,0465	0,034250309	242,2481		
		2	0,4940	0,285699993	0,0614	0,037942998	268,3661	**264,8603101**	
		3	0,4940	0,29460001	0,0703	0,040148702	283,9667		**21,07908841**
NaOCl	B	1	0,5354	0,334000003	0,1097	0,049913261	329,5916		
		2	0,5354	0,344000089	0,1197	0,052391597	345,9568	**333,955642**	
		3	0,5354	0,331999987	0,1077	0,049417594	326,3186		**10,52132813**
	C	1	0,47474	0,317699999	0,0934	0,045873606	335,7603	**338,1789777**	**2,449377333**

		2	0,47474	0,3204	0,0961	0,046542752	340,6580		
		3	0,47474	0,319000113	0,0947	0,046195816	338,1187		
NEW	A	1	0,4904	0,307599992	0,0833	0,043370507	308,6876	**326,4445242**	
		2	0,4904	0,328399986	0,1041	0,0485254	345,3774		
		3	0,4904	0,316999996	0,0927	0,045700124	325,2686		**18,37310688**
	B	1	0,5241	0,408199996	0,1839	0,068302354	459,2718	**464,8265755**	
		2	0,5241	0,413300008	0,1890	0,069566298	467,7706		
		3	0,5241	0,413100004	0,1888	0,06951673	467,4373		**4,813502592**
	C	1	0,5429	0,316199999	0,0919	0,045501859	296,9397	**296,5622726**	
		2	0,5429	0,313199997	0,0889	0,044758364	292,0877		
		3	0,5429	0,318499992	0,0942	0,04607187	300,6595		**4,298332273**
UV-C	A	1	0,5249	0,31099999	0,0867	0,044213133	296,9077	**324,1464877**	
		2	0,5249	0,335100006	0,1108	0,050185876	337,0169		
		3	0,5249	0,335999995	0,1117	0,050408922	338,5148		**23,60133208**
	B	1	0,4893	0,27759999	0,0533	0,035935562	256,2640	**261,3892255**	
		2	0,4893	0,292899996	0,0686	0,039727385	283,3042		
		3	0,4893	0,270999956	0,0467	0,034299866	244,5995		**19,85487328**
	C	1	0,48418	0,319500009	0,0952	0,046319706	333,3186	**342,4139274**	
		2	0,48418	0,326799989	0,1025	0,04812887	346,3374		
		3	0,48418	0,327499986	0,1032	0,048302352	347,5858		**7,901492235**
NEW+ UV-C	A	1	0,5041	0,319799989	0,0955	0,04639405	322,4943	**328,6387**	
		2	0,5041	0,317599989	0,0933	0,045848821	318,7043		
		3	0,5041	0,332700014	0,1084	0,049591082	344,7175		**14,0530078**
	B	1	0,5391	0,340999866	0,1167	0,051648047	339,0607	**339,7658**	
		2	0,5391	0,348100007	0,1238	0,053407685	350,6124		
		3	0,5391	0,335200012	0,1109	0,05021066	329,6245		**10,51171741**
	C	1	0,4906	0,275400002	0,0511	0,035390336	251,8009	**258,3251**	
		2	0,4906	0,270799994	0,0465	0,034250309	243,6896		
		3	0,4906	0,291099995	0,0668	0,039281288	279,4848		**18,76829224**

Day 5									
		Rep (plat)	**Weight (g)**	**Abs.**	**Abs - Blank**	**mg AA/ml sample**	**mg AAE / kg fw**	**Mean**	**St. Dev.**
Control	**A**	**1**	**0,5146**	**0,440000**	**0,2157**	**0,0761834**	**520,3151**	**528,9476**	
		2	**0,5146**	**0,454900**	**0,2306**	**0,0798761**	**545,5353**		
		3	**0,5146**	**0,440400**	**0,2161**	**0,0762825**	**520,9922**		**14,36941979**
	B	**1**	**0,5090**	**0,307900**	**0,0836**	**0,0434449**	**299,5049**	**299,9605401**	
		2	**0,5090**	**0,306600**	**0,0823**	**0,0431227**	**297,2838**		**2,931173292**

		3	0,5090	0,310000	0,0857	0,0439653	303,0928		
	C	1	0,5060	0,358600	0,1343	0,0560099	388,0845	395,7546317	
		2	0,5060	0,369200	0,1449	0,0586369	406,2867		
		3	0,5060	0,361400	0,1371	0,0567038	392,8927		9,432545444
NaOCl	A	1	0,5081	0,434600	0,2103	0,0748451	516,7568	517,3271472	
		2	0,5081	0,439300	0,2150	0,0760099	524,7990		
		3	0,5081	0,430900	0,2066	0,0739281	510,4257		7,203633634
	B	1	0,5000	0,381400	0,1571	0,0616605	431,6233	435,5555566	
		2	0,5000	0,392000	0,1677	0,0642875	450,0124		
		3	0,5000	0,377600	0,1533	0,0607187	425,0310		12,94659414
	C	1	0,4992	0,433500	0,2092	0,0745725	522,7245	522,4348292	
		2	0,4992	0,438000	0,2137	0,0756877	530,5415		
		3	0,4992	0,428500	0,2042	0,0733333	514,0385		8,255328042
HO2	A	1	0,49495	0,447200	0,2229	0,0779678	550,5475	546,6387154	
		2	0,49495	0,447700	0,2234	0,0780917	551,4225		
		3	0,49495	0,439999	0,2157	0,0761832	537,9461		7,540716486
	B	1	0,5105	0,420300	0,1960	0,0713011	490,3087	460,4277208	
		2	0,5105	0,392600	0,1683	0,0644362	443,1013		
		3	0,5105	0,395400	0,1711	0,0651301	447,8732		25,98741575
	C	1	0,4990	0,407000	0,1827	0,0680050	476,8524	479,227401	
		2	0,4990	0,413100	0,1888	0,0695167	487,4530		
		3	0,4990	0,405000	0,1807	0,0675093	473,3768		7,332439944
NEW	A	1	0,5160	0,247200	0,0229	0,0284015	193,5264	196,0031895	
		2	0,5160	0,238200	0,0139	0,0261710	178,3280		
		3	0,5160	0,260600	0,0363	0,0317224	216,1552		19,03482046
	B	1	0,4864	0,261100	0,0368	0,0318463	228,2670	241,9571535	
		2	0,4864	0,269320	0,0450	0,0338835	242,8690		
		3	0,4864	0,276000	0,0517	0,0355390	254,7354		13,25770048
	C	1	0,4841	0,241800	0,0175	0,0270632	194,7757	195,9588091	
		2	0,4841	0,241090	0,0168	0,0268872	193,5092		
		3	0,4841	0,244500	0,0202	0,0277323	199,5915		3,209122925
UV-C	A	1	0,51263	0,290800	0,0665	0,0392069	268,6528	265,1997957	
		2	0,51263	0,282300	0,0580	0,0371004	254,2182		
		3	0,51263	0,293200	0,0689	0,0398017	272,7284		9,726211268
	B	1	0,4840	0,276900	0,0526	0,0357621	257,4508	255,6666427	
		2	0,4840	0,269100	0,0448	0,0338290	243,5345		11,34573355

		3	0,4840	0,281700	0,0574	0,0369517	266,0146		
	C	1	0,5037	0,390900	0,1666	0,0640149	445,2827	457,5223702	
		2	0,5037	0,403000	0,1787	0,0670136	466,1419		
		3	0,5037	0,400100	0,1758	0,0662949	461,1426		10,89062154
NEW+HO2	A	1	0,5269	0,301600	0,0773	0,0418835	280,3549	297,5522	
		2	0,5269	0,311600	0,0873	0,0443618	296,9439		
		3	0,5269	0,322700	0,0984	0,0471128	315,3578		17,50935656
	B	1	0,5050	0,297000	0,0727	0,0407435	282,7841	292,5313	
		2	0,5050	0,301800	0,0775	0,0419331	291,0406		
		3	0,5050	0,309200	0,0849	0,0437670	303,7692		10,57170029
	C	1	0,4935	0,324300	0,1000	0,0475093	336,3196	333,3956	
		2	0,4935	0,326900	0,1026	0,0481537	340,8810		
		3	0,4935	0,316700	0,0924	0,0456258	322,9862		9,298868174
NEW+UV-C	A	1	0,4847	0,287900	0,0636	0,0384882	276,7071	284,0717	
		2	0,4847	0,289500	0,0652	0,0388848	279,5579		
		3	0,4847	0,298700	0,0744	0,0411648	295,9501		10,38527812
	B	1	0,5316	0,290500	0,0662	0,0391326	259,9712	262,2817	
		2	0,5316	0,295700	0,0714	0,0404213	268,5326		
		3	0,5316	0,289510	0,0652	0,0388872	258,3412		5,474478886
	C	1	0,4753	0,260300	0,0360	0,0316481	231,4045	264,7471	
		2	0,4753	0,293000	0,0687	0,0397522	290,6601		
		3	0,4753	0,282800	0,0585	0,0372243	272,1766		30,31835439
UV-C+HO2	A	1	0,4975	0,552000	0,3277	0,1039405	730,7175	723,5160	
		2	0,4975	0,543500	0,3192	0,1018340	715,9080		
		3	0,4975	0,548100	0,3238	0,1029740	723,9226		7,413093714
	B	1	0,4750	0,543000	0,3187	0,1017100	744,0888	750,8579	
		2	0,4750	0,544100	0,3198	0,1019827	746,0836		
		3	0,4750	0,553100	0,3288	0,1042131	762,4013		10,04649885
	C	1	0,4953	0,551100	0,3268	0,1037175	731,9275	739,1564	
		2	0,4953	0,555600	0,3313	0,1048327	739,7977		
		3	0,4953	0,559000	0,3347	0,1056753	745,7441		6,930562565
MIX	A	1	0,5230	0,347100	0,1228	0,0531599	358,0921	357,4243	
		2	0,5230	0,351200	0,1269	0,0541760	364,9367		
		3	0,5230	0,341800	0,1175	0,0518463	349,2441		7,867580986
	B	1	0,5007	0,342310	0,1180	0,0519727	363,3732	379,4183	
		2	0,5007	0,357000	0,1327	0,0556133	388,8269		13,96440697

		3	0,5007	0,355400	0,1311	0,0552169	386,0548		
	C	1	0,5037	0,338400	0,1141	0,0510037	354,7781		
		2	0,5037	0,340120	0,1158	0,0514300	357,7432	358,0075	
		3	0,5037	0,342300	0,1180	0,0519703	361,5013		3,369385806

Day 9									
		Rep (plat)	Weight (g)	Abs.	Abs – Blank	mg AA/ml sample	mg AAE / kg fw	Mean	St. Dev.
Control	A	1	0,4844	0,4130000	0,1887	0,069491951	499,8715		
		2	0,4844	0,4172000	0,1929	0,070532838	507,3588	504,1499732	
		3	0,4844	0,4160000	0,1917	0,07023544	505,2196		3,85656822
	B	1	0,5061	0,4966000	0,2723	0,090210658	624,9508		
		2	0,5061	0,5009000	0,2766	0,091276326	632,3334	624,8935247	
		3	0,5061	0,4922000	0,2679	0,089120198	617,3964		7,46864818
	C	1	0,4953	0,5063500	0,2821	0,092627014	653,6628		
		2	0,4953	0,5085100	0,2842	0,09316233	657,4405	651,1793551	
		3	0,4953	0,4999300	0,2756	0,091035937	642,4347		7,80508703
NaOCl	A	1	0,5101	0,5033000	0,2790	0,091871131	632,1836		
		2	0,5101	0,4984800	0,2742	0,090676581	623,9637	630,5805303	
		3	0,5101	0,5053000	0,2810	0,092366788	635,5943		5,97874534
	B	1	0,5113	0,5036180	0,2793	0,091949939	631,4567		
		2	0,5113	0,5073000	0,2830	0,092862453	637,7233	635,8046565	
		3	0,5113	0,5076000	0,2833	0,092936807	638,2339		3,77407087
	C	1	0,4976	0,5072700	0,2830	0,09285502	652,6723		
		2	0,4976	0,5087000	0,2844	0,093209413	655,1633	653,5258207	
		3	0,4976	0,5073100	0,2830	0,092864932	652,7419		1,41849835
HO2	A	1	0,5119	0,4374000	0,2131	0,075539037	518,2370		
		2	0,5119	0,4334000	0,2091	0,074547712	511,4360	521,1274743	
		3	0,5119	0,4465000	0,2222	0,077794301	533,7093		11,4144992
	B	1	0,4862	0,4365000	0,2122	0,075315989	540,0383		
		2	0,4862	0,4475000	0,2232	0,07804213	559,5855	552,3589505	
		3	0,4862	0,4463000	0,2220	0,077744734	557,4531		10,7231712
	C	1	0,5045	0,4302000	0,2059	0,073754642	512,3353		
		2	0,5045	0,4359000	0,2116	0,075167294	522,1482	514,9750136	
		3	0,5045	0,4291000	0,2048	0,073482026	510,4415		6,28393201
NEW	A	1	0,4855	0,3931000	0,1688	0,064560103	463,4897		
		2	0,4855	0,3811000	0,1568	0,061586122	442,1389	469,3018194	
		3	0,4855	0,4149000	0,1906	0,069962827	502,2769		30,4873904

	B	1	0,4864	0,3694000	0,1451	0,058686497	420,6509	416,565187	
		2	0,4864	0,3617000	0,1374	0,056778191	406,9726		
		3	0,4864	0,3702000	0,1459	0,058884761	422,0720		8,33773335
	C	1	0,5097	0,4162000	0,1919	0,07028501	483,9696	446,3692085	
		2	0,5097	0,3863000	0,1620	0,062874845	432,9446		
		3	0,5097	0,3800000	0,1557	0,061313506	422,1935		33,0036045
UV-C	A	1	0,5034	0,3331000	0,1088	0,049690209	345,8178	336,9064602	
		2	0,5034	0,3256000	0,1013	0,047831475	332,8820		
		3	0,5034	0,3251000	0,1008	0,047707561	332,0196		7,72947998
	B	1	0,4977	0,4937000	0,2694	0,089491946	628,9250	617,0234588	
		2	0,4977	0,4871000	0,2628	0,087856266	617,4299		
		3	0,4977	0,4798000	0,2555	0,086047085	604,7155		12,1098888
	C	1	0,5051	0,4839999	0,2597	0,087087958	604,3397	592,0145683	
		2	0,5051	0,4793000	0,2550	0,085923171	596,2568		
		3	0,5051	0,4672000	0,2429	0,082924415	575,4472		14,9061217
NEW+HO2	A	1	0,4945	0,3678000	0,1435	0,058289963	411,9197	401,9369	
		2	0,4945	0,3587000	0,1344	0,056034699	395,9823		
		3	0,4945	0,3598000	0,1355	0,056307311	397,9088		8,69880316
	B	1	0,5050	0,3849000	0,1606	0,062527884	433,9807	433,3500	
		2	0,5050	0,3865000	0,1622	0,062924412	436,7328		
		3	0,5050	0,3822000	0,1579	0,061858737	429,3364		3,73832784
	C	1	0,5097	0,3763000	0,1520	0,060396533	415,8794	416,0500	
		2	0,5097	0,3788000	0,1545	0,061016107	420,1456		
		3	0,5097	0,3741000	0,1498	0,059851306	412,1250		4,0130239
NEW+UV-C	A	1	0,4915	0,3016000	0,0773	0,041883522	297,5307	319,5021	
		2	0,4915	0,3249000	0,1006	0,047657994	338,5511		
		3	0,4915	0,3157400	0,0914	0,045387857	322,4246		20,6658092
	B	1	0,5234	0,3199000	0,0956	0,046418839	312,4802	295,3519	
		2	0,5234	0,3076000	0,0833	0,043370515	291,9596		
		3	0,5234	0,3014000	0,0771	0,041833952	281,6159		15,7093066
	C	1	0,4853	0,3019000	0,0776	0,041957869	301,3306	319,9005	
		2	0,4853	0,3072000	0,0829	0,04327138	310,7639		
		3	0,4853	0,3279000	0,1036	0,048401486	347,6070		24,4537203
UV-C+HO2	A	1	0,4798	0,5495000	0,3252	0,103320945	749,3460	743,7740	
		2	0,4798	0,5429999	0,3187	0,101710019	737,6626		
		3	0,4798	0,5467000	0,3224	0,102627017	744,3132		5,86034491

	B	**1**	**0,5236**	**0,5393500**	**0,3151**	**0,100805453**	**678,3768**	**685,1036**	
		2	**0,5236**	**0,5429000**	**0,3186**	**0,101685251**	**684,2975**		
		3	**0,5236**	**0,5479000**	**0,3236**	**0,102924417**	**692,6365**		**7,16394982**
	C	**1**	**0,5299**	**0,5456000**	**0,3213**	**0,102354399**	**681,8283**	**682,8737**	
		2	**0,5299**	**0,5409998**	**0,3167**	**0,101214324**	**674,2337**		
		3	**0,5299**	**0,5521000**	**0,3278**	**0,103965311**	**692,5593**		**9,20740062**
MIX	**A**	**1**	**0,5011**	**0,3335000**	**0,1092**	**0,049789343**	**347,8696**	**344,4065**	
		2	**0,5011**	**0,3336000**	**0,1093**	**0,049814131**	**348,0428**		
		3	**0,5011**	**0,3274000**	**0,1031**	**0,048277572**	**337,3071**		**6,14885996**
	B	**1**	**0,5007**	**0,2962000**	**0,0719**	**0,040545232**	**283,4765**	**295,4209**	
		2	**0,5007**	**0,3043000**	**0,0800**	**0,042552667**	**297,5117**		
		3	**0,5007**	**0,3087800**	**0,0845**	**0,04366295**	**305,2744**		**11,0483297**
	C	**1**	**0,5254**	**0,2980000**	**0,0737**	**0,040991329**	**275,0492**	**254,4842**	
		2	**0,5254**	**0,2850000**	**0,0607**	**0,037769512**	**253,4310**		
		3	**0,5254**	**0,2739000**	**0,0496**	**0,035018589**	**234,9725**		**20,0590975**

Day 15									
		Rep (plat)	**Weight (g)**	**Abs.**	**Abs - Blanco**	**mg AA/ml sample**	**mg AAE /kg fw**	**Mean**	**St. Dev.**
Control	A	1	0,4910	0,2568000	0,0325	0,030780669	218,8499	**227,4254**	
		2	0,4910	0,2546000	0,0303	0,030235444	214,9734		
		3	0,4910	0,2736000	0,0493	0,03494424	248,4528		**18,31316945**
	B	1	0,5231	0,2885000	0,0642	0,03863693	260,2213	**257,7175635**	
		2	0,5231	0,2952000	0,0709	0,040297396	271,4046		
		3	0,5231	0,2773000	0,0530	0,035861215	241,5268		**15,0954701**
	C	1	0,5224	0,3093000	0,0850	0,043791824	295,2763	**286,141131**	
		2	0,5224	0,2825000	0,0582	0,037149938	250,4919		
		3	0,5224	0,3197000	0,0954	0,04636927	312,6553		**32,07274376**
NaOCl	A	1	0,5023	0,2890000	0,0647	0,038760843	270,2610	**277,6338512**	
		2	0,5023	0,2938000	0,0695	0,039950437	278,5555		
		3	0,5023	0,2970000	0,0727	0,040743493	284,0851		**6,957968835**
	B	1	0,4815	0,2986000	0,0743	0,041140023	297,4642	**335,8119493**	
		2	0,4815	0,3389000	0,1146	0,051127634	369,6799		
		3	0,4815	0,3225000	0,0982	0,047063195	340,2918		**36,31569223**
	C	1	0,4880	0,2879000	0,0636	0,038488229	275,0962	**295,3490286**	
		2	0,4880	0,2966000	0,0723	0,040644363	290,5073		
		3	0,4880	0,3135000	0,0892	0,044832711	320,4436		**23,05818427**
HO2	A	1	0,4731	0,2712000	0,0469	0,034349443	252,1646	**291,1596969**	**33,83553195**

		2	0,4731	0,3022000	0,0779	0,042032222	308,5650		
		3	0,4731	0,3045000	0,0802	0,042602228	312,7495		
	B	1	0,4929	0,3240000	0,0997	0,047434945	336,1560		
		2	0,4929	0,3226000	0,0983	0,047087981	333,6972	**334,9851416**	
		3	0,4929	0,3234000	0,0991	0,047286248	335,1022		**1,233585743**
	C	1	0,4908	0,2807000	0,0564	0,036703842	261,0641		
		2	0,4908	0,2997000	0,0754	0,041412638	294,5564	**284,3912269**	
		3	0,4908	0,3014000	0,0771	0,041833955	297,5531		**20,25737868**
	A	1	0,4911	0,4100001	0,1857	0,06874847	488,7147		
		2	0,4911	0,4000000	0,1757	0,066270146	471,0969	**481,0803375**	
		3	0,4911	0,4070001	0,1827	0,068004976	483,4294		**9,040730471**
	B	1	0,4914	0,3914000	0,1671	0,064138788	455,7065		
NEW		2	0,4914	0,3835000	0,1592	0,06218092	441,7958	**445,141417**	
		3	0,4914	0,3813000	0,1570	0,061635691	437,9220		**9,352384226**
	C	1	0,5376	0,3900013	0,1657	0,06379215	419,7751		
		2	0,5376	0,4077000	0,1834	0,06817844	448,6385	**434,2879812**	
		3	0,5376	0,3990000	0,1747	0,066022303	434,4503		**14,43236323**
	A	1	0,4956	0,3192400	0,0949	0,046255267	326,2508		
		2	0,4956	0,3235000	0,0992	0,04731103	333,6974	**322,3585762**	
		3	0,4956	0,3083000	0,0840	0,043543994	307,1275		**13,70592305**
	B	1	0,5319	0,2850000	0,0607	0,037769525	250,7956		
UV-C		2	0,5319	0,2861000	0,0618	0,038042132	252,6058	**256,6650336**	
		3	0,5319	0,2946000	0,0703	0,040148702	266,5937		**8,645999363**
	C	1	0,5274	0,3197000	0,0954	0,046369273	310,1308		
		2	0,5274	0,3232000	0,0989	0,047236677	315,9322	**319,7998981**	
		3	0,5274	0,3337000	0,1094	0,049838911	333,3367		**12,07674815**
	A	1	0,5125	0,3027000	0,0784	0,042156138	288,9238		
		2	0,5125	0,3020000	0,0777	0,04198265	287,7347	**275,7883**	
		3	0,5125	0,2802000	0,0559	0,036579926	250,7063		**21,72974716**
	B	1	0,5050	0,3205000	0,0962	0,046567531	323,2063		
NEW+HO2		2	0,5050	0,3303000	0,1060	0,048996287	340,0633	**335,1897**	
		3	0,5050	0,3316000	0,1073	0,049318467	342,2995		**10,43796174**
	C	1	0,5272	0,3328000	0,1085	0,049615862	331,9520		
		2	0,5272	0,3284000	0,1041	0,0485254	324,6563	**312,4969**	
		3	0,5272	0,3020000	0,0777	0,041982654	280,8824		**27,62086479**
NEW+UV-C	A	1	0,4964	0,2706000	0,0463	0,034200742	240,8934	**277,8420**	**32,80824728**

		2	0,4964	0,2982000	0,0739	0,041040894	289,0721		
		3	0,4964	0,3065000	0,0822	0,043097897	303,5606		
	B	1	0,4925	0,2904000	0,0661	0,039107807	277,3280		
		2	0,4925	0,3042000	0,0799	0,042527878	301,5809	**308,2007**	
		3	0,4925	0,3293000	0,1050	0,048748448	345,6933		**34,66009038**
	C	1	0,5070	0,3168000	0,0925	0,045650558	315,7615		
		2	0,5070	0,3260000	0,1017	0,047930609	331,5325	**306,3333**	
		3	0,5070	0,2911000	0,0668	0,039281288	271,7058		**31,00771246**
	A	1	0,4833	0,2949000	0,0706	0,040223049	289,9006		
		2	0,4833	0,2984000	0,0741	0,041090463	296,1523	**310,5610**	
		3	0,4833	0,3261000	0,1018	0,047955389	345,6301		**30,53115209**
	B	1	0,4821	0,3688000	0,1445	0,058537799	422,8054		
UV-C+HO2		2	0,4821	0,3480000	0,1237	0,053382898	385,5727	**394,6422**	
		3	0,4821	0,3424000	0,1181	0,051995046	375,5485		**24,89968078**
	C	1	0,4508	0,3579999	0,1337	0,055861193	427,6083		
		2	0,4508	0,3446000	0,1203	0,052540271	402,1872	**417,6802**	
		3	0,4508	0,3557000	0,1314	0,055291199	423,2451		**13,59353662**
	A	1	0,5040	0,4175000	0,1932	0,070607187	490,8881		
		2	0,5040	0,4124000	0,1881	0,069343249	482,1007	**493,2429**	
		3	0,5040	0,4267000	0,2024	0,072887236	506,7398		**12,48722294**
	B	1	0,5149	0,3290000	0,1047	0,048674101	332,2676		
MIX		2	0,5149	0,3354000	0,1111	0,05026022	343,0951	**346,5914**	
		3	0,5149	0,3480000	0,1237	0,053382898	364,4116		**16,3547492**
	C	1	0,4965	0,3252000	0,1009	0,047732341	336,1453		
		2	0,4965	0,3016000	0,0773	0,041883527	294,9562	**312,5837**	
		3	0,4965	0,3083000	0,0840	0,043543988	306,6497		**21,22604086**

Day 19									
		Rep (plat)	**Weight (g)**	**Abs.**	**Abs-Blank**	**mg AA/ml sample**	**mg AAE/ kg fw**	**Mean**	**St. Dev**
	A	1	0,5013	0,359998531	0,1357	0,056356514	393,6187		
		2	0,5013	0,360000145	0,1357	0,056356914	393,6215	**410,5262391**	
Control		3	0,5013	0,389299989	0,1650	0,063618337	444,3385		**29,2822681**
	B	1	0,4682	0,363400006	0,1391	0,057199507	423,7064		
		2	0,4682	0,363300014	0,1390	0,057174725	423,5228	**420,2183305**	
		3	0,4682	0,357799995	0,1335	0,055811648	413,4258		**5,88322653**

	C	1	0,5049	0,376399994	0,1521	0,060421313	419,4309	386,8008486	40,6064041
		2	0,5049	0,364899993	0,1406	0,057571251	399,6464		
		3	0,5049	0,331	0,1067	0,049169765	341,3252		
NaOCl	A	1	0,5074	0,421799986	0,1975	0,07167286	495,4383	501,5313541	6,97767183
		2	0,5074	0,429800004	0,2055	0,073655516	509,1434		
		3	0,5074	0,42447	0,2002	0,072334573	500,0124		
	B	1	0,4809	0,374799995	0,1505	0,060024783	434,4776	429,095955	7,53427876
		2	0,4809	0,373600006	0,1493	0,059727388	432,3249		
		3	0,4809	0,367000014	0,1427	0,058091702	420,4853		
	C	1	0,5085	0,394000125	0,1697	0,064783179	446,9848	439,004862	8,2174171
		2	0,5085	0,389600009	0,1653	0,063692692	439,4608		
		3	0,5085	0,38440001	0,1601	0,062403969	430,5690		
HO2	A	1	0,5266	0,307299991	0,0830	0,043296157	289,9511	309,7015973	19,1960194
		2	0,5266	0,33039999	0,1061	0,049021064	328,2903		
		3	0,5266	0,319900006	0,0956	0,046418837	310,8634		
	B	1	0,5143	0,328900003	0,1046	0,04864932	332,4071	339,2926406	10,3564165
		2	0,5143	0,32999897	0,1057	0,048921679	334,2680		
		3	0,5143	0,339999676	0,1157	0,051400168	351,2028		
	C	1	0,5010	0,306800008	0,0825	0,043172246	301,6887	329,686948	25,4425061
		2	0,5010	0,326599985	0,1023	0,048079303	335,9793		
		3	0,5010	0,335500002	0,1112	0,050285007	351,3928		
NEW	A	1	0,5149	0,431899995	0,2076	0,07417596	506,3528	522,3684775	27,1558973
		2	0,5149	0,459899992	0,2356	0,08111524	553,7230		
		3	0,5149	0,432300024	0,2080	0,0742751	507,0296		
	B	1	0,4988	0,431899995	0,2076	0,07417596	520,3024	542,5539273	24,6043086
		2	0,4988	0,459899992	0,2356	0,08111524	568,9776		
		3	0,4988	0,442300024	0,2180	0,076753414	538,3818		

	C	1	0,4837	0,427299991	0,2030	0,073035934	526,0562	528,7877277	
		2	0,4837	0,430000567	0,2057	0,073705222	530,8769		
		3	0,4837	0,429190001	0,2049	0,073504338	529,4300		2,4736912
UV-C	A	1	0,4888	0,336300005	0,1120	0,050483273	360,3233	361,7974005	
		2	0,4888	0,3398	0,1155	0,051350682	366,5144		
		3	0,4888	0,335299999	0,1110	0,05023544	358,5544		4,17972865
	B	1	0,4774	0,351450002	0,1272	0,054237919	395,0711	392,87475	
		2	0,4774	0,351300001	0,1270	0,054200744	394,8003		
		3	0,4774	0,34795	0,1237	0,053370509	388,7528		3,57224359
	C	1	0,5092	0,362100005	0,1378	0,056877325	391,9755	432,4539814	
		2	0,5092	0,398600012	0,1743	0,065923176	454,3158		
		3	0,5092	0,396699995	0,1724	0,065452292	451,0707		35,0929484
NEW+HO2	A	1	0,4980	0,33129999	0,1070	0,049244112	345,8954	354,8895	
		2	0,4980	0,328299999	0,1040	0,04850062	340,6730		
		3	0,4980	0,349799991	0,1255	0,053828995	378,1000		20,2698235
	B	1	0,5043	0,350899994	0,1266	0,05410161	375,9434	406,8846	
		2	0,5043	0,386799991	0,1625	0,062998759	437,7683		
		3	0,5043	0,368900001	0,1446	0,058562578	406,9420		30,9124728
	C	1	0,5069	0,435699999	0,2114	0,075117721	519,6890	529,5192	
		2	0,5069	0,451200008	0,2269	0,078959111	546,2650		
		3	0,5069	0,437400013	0,2131	0,075539037	522,6038		14,5752712
NEW+UV-C	A	1	0,4829	0,359299988	0,1350	0,056183393	405,2208	409,3857	
		2	0,4829	0,359289999	0,1350	0,056180917	405,2030		
		3	0,4829	0,366299987	0,1420	0,057918213	417,7332		7,2291578
	B	1	0,4572	0,369700009	0,1454	0,058760845	444,3307	428,1391	
		2	0,4572	0,352380002	0,1281	0,054468403	411,8726		
		3	0,4572	0,36110000	0,1368	0,056629494	428,2141		16,2291692

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	C	1	0,5029	0,342900007	0,1186	0,052118962	363,0294		
		2	0,5029	0,348100007	0,1238	0,053407685	372,0059	**368,9562**	
		3	0,5029	0,34799999	0,1237	0,053382898	371,8333		**5,13345627**
UV-C+HO2	A	1	0,5128	0,323000014	0,0987	0,047187117	323,2428		
		2	0,5128	0,326900005	0,1026	0,048153658	329,8638	**322,0544**	
		3	0,5128	0,317000002	0,0927	0,045700125	313,0566		**8,46642339**
	B	1	0,5140	0,349099988	0,1248	0,053655512	366,8200		
		2	0,5140	0,340899992	0,1166	0,051623295	352,9266	**366,4811**	
		3	0,5140	0,356699972	0,1324	0,055539027	379,6968		**13,3883176**
	C	1	0,5087	0,357199997	0,1329	0,055662949	383,9288		
		2	0,5087	0,349269999	0,1250	0,053697646	370,3734	**370,8805**	
		3	0,5087	0,342230001	0,1179	0,051952913	358,3393		**12,8023097**
MIX	A	1	0,5209	0,345299989	0,1210	0,052713753	356,3061		
		2	0,5209	0,35213	0,1278	0,054406444	367,7475	**368,6074**	
		3	0,5209	0,360500008	0,1362	0,056480796	381,7685		**12,7529792**
	B	1	0,5208	0,3741	0,1498	0,059851302	404,6169		
		2	0,5208	0,357999957	0,1337	0,055861204	377,6423	**382,8921**	
		3	0,5208	0,351299995	0,1270	0,054200743	366,4170		**19,6335708**
	C	1	0,5023	0,3695	0,1452	0,058711277	409,3659		
		2	0,5023	0,370900005	0,1466	0,059058242	411,7852	**415,1836**	
		3	0,5023	0,378199995	0,1539	0,06086741	424,3996		**8,07247606**

2.3.2.DPPH free-Radical Scavenging Activity Assay

Day 0								
		Rep (plat)	**Weight (g)**	**Abs.**	**ΔDPPH**	**mg AAE/ kg fw**	**Mean**	**St. Dev.**
Control	A	1	0,5120	0,698560001	0,2396	157,8746520	**155,62546**	**2,22885**

		2	0,5120	0,704999948	0,2332	153,4175337		
		3	0,5120	0,701999973	0,2362	155,5841874		
	B	1	0,5027	0,698999949	0,2392	160,4969954		
		2	0,5027	0,712499988	0,2257	151,3418607	**156,47325**	**4,67699**
		3	0,5027	0,703300011	0,2349	157,5808815		
	C	1	0,5040	0,693400013	0,2448	163,8708499		
		2	0,5040	0,701000142	0,2372	158,7300702	**158,63994**	**5,27655**
		3	0,5040	0,70900002	0,2292	153,3188974		
	A	1	0,4940	0,669270003	0,2689	183,8401788		
		2	0,4940	0,658280002	0,2799	191,4243718	**189,4897934**	
		3	0,4940	0,655699999	0,2825	193,2048297		**4,973038984**
	B	1	0,5354	0,671299973	0,2669	168,3321128		
NaOCl		2	0,5354	0,66740001	0,2708	170,8153642	**170,7729288**	
		3	0,5354	0,663699982	0,2745	173,1713093		**2,419877326**
	C	1	0,47474	0,678400002	0,2598	184,7422774		
		2	0,47474	0,670799923	0,2674	190,1998666	**189,8161266**	
		3	0,47474	0,664802999	0,2734	194,5062358		**4,893277336**
	A	1	0,4904	0,627910004	0,3103	213,9417782		
		2	0,4904	0,634910011	0,3033	209,0756154	**207,6642680**	
		3	0,4904	0,648000717	0,2902	199,9754102		**7,08934307**
	B	1	0,5241	0,617510004	0,3207	206,9500143		
NEW		2	0,5241	0,603900099	0,3343	215,8027912	**210,4863621**	
		3	0,5241	0,61480999	0,3234	208,7062809		**4,687156069**
	C	1	0,5429	0,611409998	0,3268	203,6140173		
		2	0,5429	0,623460007	0,3147	196,0473245	**199,2226175**	
		3	0,5429	0,62033999	0,3178	198,0065107		**3,927199631**
	A	1	0,5249	0,633099985	0,3051	196,5093088		
		2	0,5249	0,62572	0,3124	201,3024197	**197,1327951**	
		3	0,5249	0,637600012	0,3006	193,5866568		**3,895484647**
	B	1	0,4893	0,661000252	0,2772	191,3678350		
UV-C		2	0,4893	0,659980006	0,2782	192,0786688	**194,1827151**	
		3	0,4893	0,649900074	0,2883	199,1016415		**4,274716219**
	C	1	0,48418	0,656900034	0,2813	196,2784154		
		2	0,48418	0,6460009	0,2922	203,9524494	**201,9110039**	
		3	0,48418	0,643799925	0,2944	205,5021469		**4,939122405**
NEW+UV-C	A	1	0,5041	0,608858	0,3293	221,0118018	**219,8522264**	**4,792969527**

		2	0,5041	0,618360009	0,3198	214,5858520		
		3	0,5041	0,60449996	0,3337	223,9590255		
	B	1	0,5391	0,623219991	0,3149	197,5809981		
		2	0,5391	0,618099928	0,3201	200,8187573	**201,4471536**	
		3	0,5391	0,609998703	0,3282	205,9417052		**4,215627717**
	C	1	0,4906	0,609310007	0,3289	226,7793640		
		2	0,4906	0,618600011	0,3196	220,3239079	**218,6538822**	
		3	0,4906	0,635099983	0,3031	208,8583748		**9,076464239**

Day 5								
		Rep (plat)	**Weight (g)**	**Abs.**	**ΔDPPH**	**mg AAE/kg fw**	**Mean**	**St. Dev.**
	A	1	0,5146	0,7249	0,2133	139,6274321		
		2	0,5146	0,739650003	0,1985	129,8559397	**135,21778**	
		3	0,5146	0,730119	0,2080	136,1699805		**4,95485**
	B	1	0,5090	0,739299986	0,1989	131,5190379		
Control		2	0,5090	0,746200014	0,1920	126,8976582	**130,49204**	
		3	0,5090	0,737000108	0,2012	133,0594103		**3,20669**
	C	1	0,5060	0,73051	0,2077	138,2209023		
		2	0,5060	0,720539999	0,2176	144,9380248	**140,57438**	
		3	0,5060	0,730000448	0,2082	138,5642044		**3,78293**
	A	1	0,5081	0,723500028	0,2147	142,3529618		
		2	0,5081	0,707700009	0,2305	152,9539653	**146,8930417**	
		3	0,5081	0,719000077	0,2192	145,3721981		**5,461688631**
	B	1	0,5000	0,719261	0,2189	147,5493260		
NaOCl		2	0,5000	0,729829998	0,2083	140,3431909	**145,1179624**	
		3	0,5000	0,719390001	0,2188	147,4613704		**4,135307335**
	C	1	0,4992	0,7081	0,2301	155,4077510		
		2	0,4992	0,72566	0,2125	143,4158366	**148,929205**	
		3	0,4992	0,718999994	0,2192	147,9640271		**6,05393911**
	A	1	0,49495	0,671499999	0,2667	181,9513535		
		2	0,49495	0,668689998	0,2695	183,8868110	**183,978555**	
		3	0,49495	0,6654804	0,2727	186,0975015		**2,07459601**
	B	1	0,5105	0,689499986	0,2487	164,3887622		
HO2		2	0,5105	0,6827	0,2555	168,9297628	**168,052724**	
		3	0,5105	0,679840003	0,2583	170,8396461		**3,31366472**
	C	1	0,4990	0,691200012	0,2470	167,0158494		
		2	0,4990	0,695599997	0,2426	164,0098476	**168,131726**	
		3	0,4990	0,681899989	0,2563	173,3694817		**4,77855348**

NEW	A	1	0,5160	0,670809999	0,2674	174,9845927	**169,681556**	
		2	0,5160	0,683300012	0,2549	166,7327336		
		3	0,5160	0,682400012	0,2558	167,3273427		**4,60217728**
	B	1	0,4864	0,670999998	0,2672	185,5001589	**181,970052**	
		2	0,4864	0,67781	0,2604	180,7271497		
		3	0,4864	0,679299982	0,2589	179,6828483		**3,1014321**
	C	1	0,4841	0,653719997	0,2844	198,5502717	**194,827326**	
		2	0,4841	0,665000021	0,2732	190,6067420		
		3	0,4841	0,658300021	0,2799	195,3249638		**3,99507807**
UV-C	A	1	0,51263	0,663399982	0,2748	181,0627385	**174,988882**	
		2	0,51263	0,670599997	0,2676	176,2745864		
		3	0,51263	0,683600008	0,2546	167,6293216		**6,80837345**
	B	1	0,4840	0,693799989	0,2444	170,3782477	**178,5003260**	
		2	0,4840	0,671359998	0,2668	186,1856597		
		3	0,4840	0,681649998	0,2565	178,9370707		**7,912750948**
	C	1	0,5037	0,682300007	0,2559	171,4810430	**167,0208691**	
		2	0,5037	0,689770003	0,2484	166,4252771		
		3	0,5037	0,6946	0,2436	163,1562873		**4,194214675**
NEW+HO2	A	1	0,5269	0,61656	0,3216	206,4649220	**207,4268091**	
		2	0,5269	0,600300002	0,3379	216,9852886		
		3	0,5269	0,628359997	0,3098	198,8302166		**9,115677706**
	B	1	0,5050	0,671150001	0,2670	178,5666134	**172,0634607**	
		2	0,5050	0,692400002	0,2458	164,2214281		
		3	0,5050	0,678800011	0,2594	173,4023406		**7,265709538**
	C	1	0,4935	0,6255	0,3127	214,2626958	**216,8416796**	
		2	0,4935	0,632199979	0,3060	209,6343597		
		3	0,4935	0,607600009	0,3306	226,6279832		**8,785452634**
NEW+UV-C	A	1	0,4847	0,69949	0,2387	166,1125990	**164,5605**	
		2	0,4847	0,707200027	0,2310	160,6898256		
		3	0,4847	0,698400023	0,2398	166,8792235		**3,373989538**
	B	1	0,5316	0,716249998	0,2219	140,7094451	**138,3752**	
		2	0,5316	0,717340001	0,2208	140,0104383		
		3	0,5316	0,72608	0,2121	134,4055752		**3,455475746**
	C	1	0,4753	0,718699973	0,2195	155,6194451	**155,6988**	
		2	0,4753	0,713180001	0,2250	159,5786469		
		3	0,4753	0,7238882	0,2143	151,8981872		**3,840844059**

UV-C+HO2	A	1	0,4975	0,655900002	0,2823	191,7085494	**184,1252**	
		2	0,4975	0,683099985	0,2551	173,0699129		
		3	0,4975	0,661899984	0,2763	187,5970951		**9,792359708**
	B	1	0,4750	0,672259998	0,2659	180,4979532	**190,0358**	
		2	0,4750	0,664987666	0,2732	194,2672337		
		3	0,4750	0,66349	0,2747	195,3421135		**8,277454821**
	C	1	0,4953	0,680002384	0,2582	183,4911199	**177,1273**	
		2	0,4953	0,679990001	0,2582	175,9792118		
		3	0,4953	0,685900009	0,2523	171,9114237		**5,874592587**
MIX	A	1	0,5230	0,624199986	0,3140	203,0245251	**195,6153**	
		2	0,5230	0,64349997	0,2947	190,4441427		
		3	0,5230	0,639000058	0,2992	193,3773378		**6,582019427**
	B	1	0,5007	0,64380002	0,2944	190,2485601	**199,1655**	
		2	0,5007	0,636800029	0,3014	203,4878320		
		3	0,5007	0,636400023	0,3018	203,7601826		**7,723518603**
	C	1	0,5037	0,637199974	0,3010	203,2155236	**200,4007**	
		2	0,5037	0,645000021	0,2932	196,7260386		
		3	0,5037	0,638300021	0,2999	201,2606647		**3,329107094**

Day 9								
		Rep (plat)	**Weight (g)**	**Abs.**	**ΔDPPH**	**mg AAE /kg fw**	**Mean**	**St. Dev.**
Control	A	1	0,484	0,800400	0,1378	95,1974274	**81,47380**	**12,17451**
		2	0,484	0,825900	0,1123	77,2511399		
		3	0,484	0,833400	0,1048	71,9728250		
	B	1	0,506	0,827520	0,1106	72,8476296	**72,62759**	**7,00130**
		2	0,506	0,817620	0,1205	79,5162730		
		3	0,506	0,838400	0,0998	65,5188588		
	C	1	0,495	0,816330	0,1218	82,1380140	**74,92705**	**7,54624**
		2	0,495	0,825890	0,1123	75,5579809		
		3	0,495	0,838200	0,1000	67,0851532		
NaOCl	A	1	0,510	0,769500	0,1687	111,0521930	**108,579432**	
		2	0,510	0,772500	0,1657	109,0472675		
		3	0,510	0,777600	0,1606	105,6388370		**2,73683355**
	B	1	0,511	0,816600	0,1216	79,3876482	**77,5207547**	
		2	0,511	0,821000	0,1172	76,4539557		
		3	0,511	0,820600	0,1176	76,7206601		**1,62226734**
	C	1	0,498	0,760800	0,1774	119,8023289	**113,590692**	
		2	0,498	0,768900	0,1693	114,2529626		
		3	0,498	0,779900	0,1583	106,7167839		**6,56786295**

HO2	A	1	0,512	0,701300	0,2369	156,0807287		
		2	0,512	0,690000	0,2482	163,6059202	**160,764624**	
		3	0,512	0,691500	0,2467	162,6072230		**4,08699217**
	B	1	0,486	0,723600	0,2146	148,6949017		
		2	0,486	0,729500	0,2087	144,5579930	**148,554668**	
		3	0,486	0,718300	0,2199	152,4111101		**3,92843617**
	C	1	0,505	0,725640	0,2125	141,9227041		
		2	0,505	0,725850	0,2123	141,7807980	**141,571320**	
		3	0,505	0,726990	0,2112	141,0104594		**0,49087495**
NEW	A	1	0,486	0,739000	0,1992	138,0956962		
		2	0,486	0,748200	0,1900	131,6356272	**136,831765**	
		3	0,486	0,735200	0,2030	140,7639731		**4,6935921**
	B	1	0,486	0,769280	0,1689	116,6174581		
		2	0,486	0,772270	0,1659	114,5218194	**117,713169**	
		3	0,486	0,761600	0,1766	122,0002282		**3,857730668**
	C	1	0,510	0,769510	0,1687	111,1326704		
		2	0,510	0,768180	0,1700	112,0222316	**113,326503**	
		3	0,510	0,761000	0,1772	116,8246078		**3,06192444**
UV-C	A	1	0,503	0,781000	0,1572	104,7422932		
		2	0,503	0,812300	0,1259	83,5455165	**98,8279660**	
		3	0,503	0,775900	0,1623	108,1960882		**13,3471765**
	B	1	0,498	0,750001	0,1882	127,1752565		
		2	0,498	0,736927	0,2012	136,1305261	**130,845603**	
		3	0,498	0,747000	0,1912	129,2310272		**4,690880029**
	C	1	0,505	0,739850	0,1983	132,1633063		
		2	0,505	0,740000	0,1982	132,0620511	**132,320774**	
		3	0,505	0,739000	0,1992	132,7369637		**0,36396942**
NEW+HO2	A	1	0,495	0,808200	0,1300	87,8757362		
		2	0,495	0,793520	0,1446	97,9961463	**97,5687212**	
		3	0,495	0,780700	0,1575	106,8342810		**9,48649695**
	B	1	0,505	0,784000	0,1542	102,3852519		
		2	0,505	0,787600	0,1506	99,9550048	**98,2448338**	
		3	0,505	0,798800	0,1394	92,3942447		**5,21042899**
	C	1	0,510	0,746000	0,1922	126,8571421		
		2	0,510	0,775600	0,1626	107,0594238	**119,67825**	
		3	0,510	0,748600	0,1896	125,1181816		**10,9627579**

NEW+UV-C	A	1	0,492	0,764700	0,1735	118,5841185	**120,5192**	
		2	0,492	0,762030	0,1761	120,4360557		
		3	0,492	0,759000	0,1792	122,5375676		**1,97803704**
	B	1	0,523	0,763750	0,1744	111,9754658	**110,8992**	
		2	0,523	0,765897	0,1723	110,5770473		
		3	0,523	0,766560	0,1716	110,1452117		**0,95672066**
	C	1	0,485	0,781480	0,1567	108,3116410	**110,5258**	
		2	0,485	0,770784	0,1674	115,8252705		
		3	0,485	0,782720	0,1554	107,4405799		**4,61006888**
UV-C+HO2	A	1	0,480	0,704580	0,2336	164,1924733	**160,9145**	
		2	0,480	0,717000	0,2212	155,3677695		
		3	0,480	0,706000	0,2322	163,1834000		**4,83007430**
	B	1	0,524	0,720000	0,2182	140,4177478	**142,4144**	
		2	0,524	0,720500	0,2177	140,0922155		
		3	0,524	0,710300	0,2279	146,7332985		**3,74379786**
	C	1	0,530	0,739400	0,1988	126,2674071	**124,3996**	
		2	0,530	0,740610	0,1976	125,4889504		
		3	0,530	0,746900	0,1913	121,4422969		**2,5904661**
MIX	A	1	0,501	0,692700	0,2455	165,2954293	**171,5136**	
		2	0,501	0,672280	0,2659	179,1876120		
		3	0,501	0,685700	0,2525	170,0576994		**7,05959468**
	B	1	0,501	0,661300	0,2769	186,8066789	**186,6160**	
		2	0,501	0,659740	0,2784	187,8688089		
		3	0,501	0,663700	0,2745	185,1725964		**1,35817939**
	C	1	0,525	0,675100	0,2631	169,0703075	**165,9991**	
		2	0,525	0,680500	0,2577	165,5664978		
		3	0,525	0,683900	0,2543	163,3603994		**2,87942716**

Day 15								
		Rep (plat)	**Weight (g)**	**Abs.**	**ΔDPPH**	**mg AAE/kg fw**	**Mean**	**St. Dev.**
Control	A	1	0,4910	0,8827	0,0555	36,7755951	**25,59712**	
		2	0,4910	0,9094	0,0288	18,2373891		
		3	0,4910	0,9043	0,0339	21,7783668		**9,84141**
	B	1	0,5231	0,8872	0,0510	31,5861859	**29,87002**	
		2	0,5231	0,8969	0,0413	25,2646102		
		3	0,5231	0,8854	0,0528	32,7592669		**4,03130**
	C	1	0,5224	0,8984	0,0398	24,3195664	**21,64617**	
		2	0,5224	0,9047	0,0335	20,2148369		**2,31717**

		3	0,5224	0,9044	0,0338	20,4040923		
NaOCl	A	1	0,5023	0,8630	0,0752	49,3185970	**49,1677001**	
		2	0,5023	0,8636	0,0746	48,9045924		
		3	0,5023	0,8631	0,0751	49,2799109		**0,228677497**
	B	1	0,4815	0,8745	0,0637	43,3210702	**45,9124040**	
		2	0,4815	0,8680	0,0702	47,9019244		
		3	0,4815	0,8700	0,0682	46,5142173		**2,3489765**
	C	1	0,4880	0,8950	0,0432	28,4091854	**33,6298783**	
		2	0,4880	0,8904	0,0478	31,6365571		
		3	0,4880	0,8772	0,0610	40,8438924		**6,452556214**
HO2	A	1	0,4731	0,8584	0,0798	55,6772632	**57,9831335**	
		2	0,4731	0,8620	0,0762	53,0831538		
		3	0,4731	0,8452	0,0930	65,1889836		**6,373818099**
	B	1	0,4929	0,8590	0,0792	53,0278410	**55,9559024**	
		2	0,4929	0,8473	0,0909	61,1203582		
		3	0,4929	0,8580	0,0802	53,7195079		**4,485900563**
	C	1	0,4908	0,8597	0,0784	52,7476753	**48,5706507**	
		2	0,4908	0,8700	0,0681	45,5860697		
		3	0,4908	0,8675	0,0707	47,3782069		**3,726739883**
NEW	A	1	0,4911	0,8543	0,0839	56,4826566	**58,7734402**	
		2	0,4911	0,8467	0,0915	61,7583893		
		3	0,4911	0,8520	0,0862	58,0792746		**2,705501457**
	B	1	0,4914	0,8167	0,1215	82,5332066	**82,0244549**	
		2	0,4914	0,8162	0,1220	82,8800783		
		3	0,4914	0,8194	0,1188	80,6600798		**1,194244313**
	C	1	0,5376	0,8330	0,1052	65,1041711	**68,2748381**	
		2	0,5376	0,8303	0,1079	66,8163457		
		3	0,5376	0,8207	0,1175	72,9039977		**4,099356514**
UV-C	A	1	0,4956	0,8809	0,0573	37,6724250	**40,4927149**	
		2	0,4956	0,8731	0,0651	43,0378516		
		3	0,4956	0,8764	0,0618	40,7678681		**2,693275465**
	B	1	0,5319	0,9125	0,0257	14,8481464	**14,8908769**	
		2	0,5319	0,9117	0,0265	15,3609082		
		3	0,5319	0,9131	0,0251	14,4635761		**0,450189585**
	C	1	0,5274	0,9089	0,0293	17,3018648	**16,9786683**	
		2	0,5274	0,9191	0,0191	10,7086380		**6,114841224**

		3	0,5274	0,9002	0,0380	22,9255020		
NEW+HO2	A	1	0,5125	0,8189	0,1193	77,6718551	**79,1352615**	
		2	0,5125	0,8129	0,1253	81,6629742		
		3	0,5125	0,8183	0,1199	78,0709551		**2,198139872**
	B	1	0,5050	0,8252	0,1130	74,5724746	**73,0783182**	
		2	0,5050	0,8294	0,1088	71,7371793		
		3	0,5050	0,8276	0,1105	72,9253006		**1,423827823**
	C	1	0,5272	0,8387	0,0995	62,7026391	**63,6510356**	
		2	0,5272	0,8318	0,1064	67,1644387		
		3	0,5272	0,8412	0,0970	61,0860288		**3,148231006**
NEW+UV-C	A	1	0,4964	0,8711	0,0671	44,3419956	**46,2718**	
		2	0,4964	0,8675	0,0706	46,7868719		
		3	0,4964	0,8662	0,0719	47,6865332		**1,730738864**
	B	1	0,4925	0,8654	0,0727	48,6151439	**50,0775**	
		2	0,4925	0,8592	0,0790	52,9441704		
		3	0,4925	0,8654	0,0728	48,6732874		**2,482750234**
	C	1	0,5070	0,8601	0,0781	50,8093907	**46,0579**	
		2	0,5070	0,8734	0,0648	41,8667607		
		3	0,5070	0,8680	0,0702	45,4976449		**4,497565925**
UV-C+HO2	A	1	0,4833	0,8217	0,1165	80,3895439	**82,8396**	
		2	0,4833	0,8041	0,1341	92,8041998		
		3	0,4833	0,8289	0,1093	75,3249519		**8,993496555**
	B	1	0,4821	0,8187	0,1195	82,7393491	**84,1135**	
		2	0,4821	0,8139	0,1242	86,0911621		
		3	0,4821	0,8176	0,1206	83,5101251		**1,755491252**
	C	1	0,4508	0,8200	0,1182	87,4707712	**87,2691**	
		2	0,4508	0,8205	0,1177	87,0926685		
		3	0,4508	0,8203	0,1179	87,2439110		**0,190307419**
MIX	A	1	0,5040	0,7824	0,1558	103,6706602	**97,4026**	
		2	0,5040	0,7946	0,1436	95,4184713		
		3	0,5040	0,7980	0,1402	93,1187093		**5,548741513**
	B	1	0,5149	0,7761	0,1620	105,6226793	**103,9176**	
		2	0,5149	0,7851	0,1531	99,6883880		
		3	0,5149	0,7749	0,1633	106,4417008		**3,685418217**
	C	1	0,4965	0,7726	0,1656	111,9655883	**115,6779**	
		2	0,4965	0,7656	0,1725	116,7513575		**3,308904452**

		3	0,4965	0,7633	0,1748	118,3168635		

Day 19								
		Rep (plate)	**Weight(g)**	**Abs.**	**ΔDPPH**	**mg AAE/kg fw**	**Mean**	**St. Dev.**
Control	A	1	0,5013	1,05720	-0,1190	-82,6487417	**-87,47483**	**6,77720**
		2	0,5013	1,06000	-0,1218	-84,5528938		
		3	0,5013	1,07569	-0,1375	-95,2228673		
	B	1	0,4682	1,07983	-0,1417	-104,9692157	**-111,35246**	**6,62720**
		2	0,4682	1,09800	-0,1598	-118,1992829		
		3	0,4682	1,08796	-0,1498	-110,8888885		
	C	1	0,5049	1,02544	-0,0873	-60,6150567	**-59,68328**	**8,25673**
		2	0,5049	1,03554	-0,0974	-67,4345880		
		3	0,5049	1,01120	-0,0730	-51,0001856		
NaOCl	A	1	0,5074	0,95900	-0,0208	-15,6770629	**-16,9222697**	
		2	0,5074	0,96556	-0,0274	-20,0845589		
		3	0,5074	0,95800	-0,0198	-15,0051871		**2,759150109**
	B	1	0,4809	0,93022	0,0079	3,8611340	**0,5104084**	
		2	0,4809	0,92715	0,0110	6,0374486		
		3	0,4809	0,94747	-0,0093	-8,3673574		**7,764993897**
	C	1	0,5085	0,93700	0,0012	-0,8931122	**-1,0679408**	
		2	0,5085	0,92850	0,0097	4,8046858		
		3	0,5085	0,94628	-0,0081	-7,1153960		**5,961963711**
HO2	A	1	0,5266	0,87000	0,0682	42,5111407	**39,0001960**	
		2	0,5266	0,87839	0,0598	37,0796419		
		3	0,5266	0,87788	0,0603	37,4098053		**3,045045387**
	B	1	0,5143	0,86156	0,0766	49,1185608	**43,3432869**	
		2	0,5143	0,87607	0,0621	39,5012049		
		3	0,5143	0,87319	0,0650	41,4100950		**5,091788161**
	C	1	0,5010	0,90000	0,0382	24,2678007	**25,5051966**	
		2	0,5010	0,90445	0,0337	21,2416134		
		3	0,5010	0,89010	0,0481	31,0061756		**4,998502787**
NEW	A	1	0,5149	0,95380	-0,0156	-12,0058554	**-14,4114404**	
		2	0,5149	0,95690	-0,0187	-14,0583258		
		3	0,5149	0,96160	-0,0234	-17,1701401		**2,600187804**
	B	1	0,4988	0,96757	-0,0294	-21,8045967	**-20,6563860**	
		2	0,4988	0,96480	-0,0266	-19,9114162		
		3	0,4988	0,96530	-0,0271	-20,2531451		**1,008952665**
	C	1	0,4837	0,97910	-0,0409	-30,6140980	**-29,3078305**	**4,595978383**

		2	0,4837	0,97000	-0,0318	-24,2001187		
		3	0,4837	0,98264	-0,0445	-33,1092749		
UV-C	A	1	0,4888	0,92630	0,0119	6,5327031	**8,9039565**	
		2	0,4888	0,92300	0,0152	8,8341077		
		3	0,4888	0,91940	0,0188	11,3450587		**2,406938051**
	B	1	0,4774	0,92590	0,0123	6,9743257	**8,5453492**	
		2	0,4774	0,92880	0,0094	4,9034718		
		3	0,4774	0,91640	0,0218	13,7582500		**4,631723273**
	C	1	0,5092	0,92370	0,0145	8,0116777	**10,7343216**	
		2	0,5092	0,91710	0,0211	12,4303985		
		3	0,5092	0,91810	0,0201	11,7608886		**2,381523312**
NEW+HO2	A	1	0,4980	0,91360	0,0246	15,1058869	**14,2547544**	
		2	0,4980	0,92552	0,0126	6,9459734		
		3	0,4980	0,90541	0,0328	20,7124028		**6,922569192**
	B	1	0,5043	0,89260	0,0456	29,1132732	**22,5923079**	
		2	0,5043	0,90670	0,0315	19,5816118		
		3	0,5043	0,90744	0,0307	19,0820385		**5,652843125**
	C	1	0,5069	0,91628	0,0219	13,0382606	**17,3155972**	
		2	0,5069	0,91574	0,0224	13,4014324		
		3	0,5069	0,89774	0,0404	25,5070988		**7,096372081**
NEW+UV-C	A	1	0,4829	0,91865	0,0195	12,0096121	**10,4914**	
		2	0,4829	0,92377	0,0144	8,3955752		
		3	0,4829	0,91999	0,0182	11,0688665		**1,874955626**
	B	1	0,4572	0,92700	0,0112	6,4622667	**3,4051**	
		2	0,4572	0,94350	-0,0053	-5,8408676		
		3	0,4572	0,92280	0,0154	9,5939763		**8,158932996**
	C	1	0,5029	0,92450	0,0137	7,5697586	**9,3661**	
		2	0,5029	0,91835	0,0198	11,7387393		
		3	0,5029	0,92270	0,0155	8,7899515		**2,143385953**
UV-C+HO2	A	1	0,5128	0,85760	0,0806	51,8986855	**44,7410**	
		2	0,5128	0,87500	0,0632	40,3309887		
		3	0,5128	0,87250	0,0657	41,9931835		**6,254244845**
	B	1	0,5140	0,86410	0,0741	47,4664182	**35,8155**	
		2	0,5140	0,89000	0,0482	30,2888303		
		3	0,5140	0,89090	0,0473	29,6913738		**10,09437701**
	C	1	0,5087	0,86242	0,0757	49,0868073	**48,8858**	**1,006998209**

		2	0,5087	0,86435	0,0738	47,7934045		
		3	0,5087	0,86139	0,0768	49,7770681		
MIX	A	1	0,5209	0,82310	0,1151	73,6705967	**88,0033**	**13,48428817**
		2	0,5209	0,79830	0,1399	89,9012091		
		3	0,5209	0,78220	0,1560	100,4380743		
	B	1	0,5208	0,84700	0,0912	58,0399745	**59,1027**	**3,611452897**
		2	0,5208	0,83923	0,0989	63,1262229		
		3	0,5208	0,84990	0,0883	56,1417775		
	C	1	0,5023	0,81760	0,1206	80,1313952	**80,9685**	**1,277572797**
		2	0,5023	0,81730	0,1209	80,3349974		
		3	0,5023	0,81420	0,1240	82,4389811		